Initiatives for Addressing Environmental Antimicrobial Resistance: Current Situation and Challenges

This white paper includes knowledge gaps identified through the International Environmental Antimicrobial Resistance (AMR) Forum, a meeting hosted by the U.S. Centers for Disease Control and Prevention, the U.K. Science and Innovation Network, and the Wellcome Trust in April 2018. This scientific meeting gathered international technical experts, government officials, and key partners to outline the current knowledge of how resistant microbes and antimicrobials from multiple sources—human and animal waste, antimicrobial manufacturing, and the use of antimicrobials as pesticides—contributes to the presence of resistant microbes and antimicrobials in the environment and the potential impact of the affected environment on human health.

This white paper supports an executive summary, *Initiatives for Addressing Environmental Antimicrobial Resistance: Executive Summary*, which was drafted by the meeting co-hosts and published alongside this white paper in 2018, available online at [URL, tbd].

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Graphics in this report were selected from illustrated minutes produced at the International Environmental AMR Forum by Sam Bradd, Drawing Change.

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Acronyms

3GCs	third-generation cephalosporins
3GCREC	third-generation cephalosporin resistant *Escherichia coli*
ADI	acceptable daily intakes
AMR	antimicrobial resistance
AOEL	acceptable operator exposure level
APIs	active pharmaceutical ingredients
ARGs	antimicrobial-resistant genes
ARfD	acute reference doses
CDC	U.S. Centers for Disease Control and Prevention
CRE	carbapenem-resistant Enterobacteriaceae
DNA	deoxyribonucleic acid
ECDC	European Centers for Disease Control
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
ESBL	extended-spectrum beta-lactamase
FDA	U.S. Food and Drug Administration
IPM	integrated pest management
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC-MS/MS	liquid chromatography-tandem mass spectrometry
MALDI-ToF MS	matrix-assisted laser desorption ionization-time of flight mass spectrometry
MS	mass spectrometry
MIC	minimum inhibitory concentration
MLST	multi-locus sequence typing
MRSA	methicillin-resistant *Staphylococcus aureus*
MRL	maximum residue level
MS/MS	tandem mass spectrometry
NARMS	U.S. National Antimicrobial Resistance Monitoring System

NoAEL	No observed adverse effect level
PCR	polymerase chain reaction
PFGE	pulse-field gel electrophoresis
qPCR	quantitative polymerase chain reaction
U.K.	United Kingdom
U.S.	United States
USDA	U.S. Department of Agriculture
USGA	U.S. Geological Survey
UV	ultraviolet
VRE	vancomycin-resistant Enterococci
WGS	whole genome sequencing
WHO	World Health Organization
WWTPs	wastewater treatment plants

Introduction

The U. S Centers for Disease Control and Prevention, the U.K. Science and Innovation Network, and the Wellcome Trust cohosted the International Environmental Antimicrobial Resistance (AMR) Forum in Vancouver, B.C. on 4-5 April 2018. This scientific meeting gathered international technical experts, government officials, and key partners to outline the current knowledge of how resistant microbes and antimicrobials from multiple sources—human and animal waste, antimicrobial manufacturing, and the use of antimicrobials as pesticides—contributes to the presence of resistant microbes and antimicrobials in the environment and the potential impact of the affected environment on human health. Scientific gaps in knowledge were identified in order to understand the potential risk to human health and steps needed to mitigate this risk, as well as proposed pathways to address these gaps.

AMR—when microbes develop the ability to defeat the drugs designed to combat them—is a threat to public health and a priority across the globe. Pathogenic antimicrobial-resistant bacteria and fungi is of particular concern because these microbes can cause infections in humans that are difficult, and sometimes impossible, to treat. This report, drafted by the assembled technical experts in this field, highlights data identifying the potential for the environment (waterways and soils) to be a source of pathogenic AMR that could affect human health. The full extent to which pathogenic AMR and antimicrobials found in the environment from human activity is not well understood, including how resistance spreads and the specific risks to human health. The report also highlights significant knowledge gaps, which include the extent of environmental contamination, source of contamination, and types of contamination that are most risky for human health, as well as which measures are most important for mitigating any risks.

More research is needed to guide action, address knowledge gaps, and evaluate the potential risk antimicrobials and resistant microbes in the environment poses to human health and the broader environmental ecosystem. This report is intended as a roadmap for stakeholders including researchers, non-governmental organizations, and countries to work to address knowledge gaps and improve national and international understanding on how to best evaluate and address AMR in the environment. Stakeholders can work to understand their local situation, determine what action is needed, and move towards reducing identified risks to human health. As we improve local, national and international understanding of AMR in the environment, and as we work collaboratively to enhance collective scientific understanding, we will be able to better identify best practices, recommendations, and actions that are most significant and can be considered for wider adoption.

Human and Animal Contamination

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Summary

- Waste (i.e., feces) from people and animals can carry antimicrobials that are important in human medicine and antimicrobial-resistant microbes (including pathogens). Without proper handling of this waste (e.g., implementing basic sanitation strategies), the environment may become contaminated with antimicrobials and antimicrobial-resistant microbes.
- The connection between waste and antimicrobials and AMR in the environment, and its impact on human health, is not well understood. However, scientific evidence shows that antimicrobials and resistance do spread in the environment and people exposed to AMR pathogens like Methicillin-resistant *Staphylococcus aureus* (MRSA) and ESBL-producing bacteria in environmental waters are at increased risk of infection from this exposure.
- Basic sanitation, which includes access to facilities for disposing of human waste safely and the ability to maintain hygienic conditions, is critically important for preventing many diseases.

Human Waste

- Inadequate sanitation infrastructure around the world means that only a portion of human sewage is appropriately treated. Globally, the majority of human waste is discharged directly into the environment without treatment. If waste carries antimicrobial-resistant pathogens, then there is an increased risk of infections for people exposed to these pathogens in the environment. Increased access to sanitation globally can mitigate this potential risk.

- Wastewater treatment plants (WWTPs), or other sanitation strategies like septic systems, are essential for reducing fecal bacteria, including resistant bacteria, from wastewater. However, when levels of bacteria are high, these sanitation strategies may not be sufficient. Assessments of environmental waters for AMR pathogens can help to identify insufficient sanitation strategies.
- A main source of antimicrobials and antimicrobial-resistant bacteria in WWTP influent are healthcare facilities. Some of the most resistant infections occur in inpatients, who stay in a hospital while undergoing treatment and are commonly administered antimicrobials. Resistant microbes can persist and grow within the healthcare facility plumbing system, such as sinks drains. This reservoir of AMR is known to cause infections in hospitalized patients in some cases.
- Levels of antimicrobial-resistant microbes in sewage waste from the general population varies geographically, but when the levels are high and the sanitation infrastructure is insufficient, this may be a source of antimicrobial-resistant microbes in the environment.
- Studies have found detectable levels of resistant bacteria in surface waters (rivers, coastal waters) and people who were exposed to these microbes through interaction with contaminated water became ill.

Waste on Farms

- When antimicrobials are used in food animals, the animal manure can carry both antimicrobials and resistant bacteria. It is not known how long resistant microbes remain in manure and, subsequently, in the environment.
- Animal manure might be treated before it is used as fertilizer (e.g., composting). If used properly, treatments can be effective in reducing environmental exposure to AMR.
- Human waste produced from wastewater treatment facilities (biosolids) can be used on agricultural land and may contain antimicrobials and antimicrobial-resistant microbes. The consequences of these contaminants in agriculture are unknown.
- Runoff from livestock production or areas with manure applied can contaminate nearby surface and groundwater resources with resistant bacteria. The risk from runoff is poorly understood.

Aquaculture

- Antimicrobials are administered worldwide in aquaculture (the farming of fish and seafood), but estimates of antimicrobial use in aquaculture are difficult to determine.
- Antimicrobials are also used in large quantities to support rearing ornamental fish (pets) and other aquatic species not meant for eating.
- More information is needed on antimicrobial use in aquaculture generally, including the quantities and types used.

Addressing Knowledge Gaps

Scientific review suggests that the following actions could improve understanding and guide action. Unless specified, these apply to both human and animal waste:

Assessing Environmental Waters

- Assess where and how much resistant microbes are present in environmental waters to better understand the risk of AMR to human health
- Conduct studies to understand the drivers of AMR in recreational and drinking water, including identifying sources of resistant pathogens (human or animal) and selective pressures driving amplification and transmission of AMR in these waters
- Evaluate sampling strategies and testing methods to measure AMR in environmental waters to identify best practices

Assessing and Improving Sanitation & Wastewater Treatment

- Evaluate the need for on-site pretreatment of wastewater for facilities that may contribute AMR in the environment (e.g., hospitals) by conducting studies of the environment near waste discharge and assessing the impact of approaches to limit AMR and antimicrobial discharge
- Conduct studies to evaluate the effectiveness of existing wastewater treatment processing for removal of antimicrobial-resistant microbes and antimicrobials from wastewater before discharge into environmental waters. Investigate and identify factors that result in treatment inefficiencies and failures (e.g., ineffective processing methods or infrastructure failures)
- Improve sanitation globally by conducting research to identify efficient and affordable wastewater processing methods that are easily implemented where processing doesn't currently exist or as enhancements to existing processing where levels of AMR are high

Assessing Environmental AMR from Agriculture

- Conduct research to identify and develop alternatives to antimicrobials to prevent and control disease on the farm and in aquaculture
- Evaluate methods for treating animal manure and human waste biosolids when using these as fertilizers on the farm to prevent environmental contamination with AMR and antimicrobials

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~~Conduct studies to understand the drivers of AMR in recreational and drinking water, including identifying sources of resistant pathogens (human or animal), selective pressures driving amplification (e.g., co-selecting agents like heavy metals), and identifying mechanisms for amplification and transmission (e.g., horizontal gene transfer).~~

~~Improve sanitation globally by conducting research to identify efficient and affordable wastewater processing methods that are easily implemented where processing doesn't currently exist or as enhancements to existing processing where levels of AMR are high.~~

~~Understand the risk of antimicrobial-resistant microbes in environmental waters by conducting studies to assess where bacteria are present and how much is there. Assessments should prioritize microbial targets described in national and international AMR threat lists.~~

~~Conduct evaluation studies of potential environmental contamination between waste influent and waste processing (e.g., WWTP) to help to determine if on-site pre-treatment of wastewater is needed. Studies are most needed to evaluate the benefits of preventing environmental contamination through on-site pre-treatment methods to manage hospital waste.~~

~~Conduct studies to evaluate the presence of antimicrobial-resistant microbes and antimicrobials in post-processing waste streams to measure the effectiveness of treatment processes in areas where AMR and antimicrobial levels in wastewater are likely to be high, such as hospitals, sewage systems, and farms.~~

~~Investigate and identify factors that improve wastewater treatment efficiencies and those factors that contribute to inefficiencies and failures (e.g., ineffective processing method or infrastructure failures).~~
~~Conduct research to identify and develop alternatives to antibiotics to prevent and control disease in aquaculture.~~

Background Statement

Bacteria and fungi that cause infections in people and animals are becoming increasingly resistant to antimicrobials. In addition to causing infections, these organisms can colonize (be present in) people or animals without causing disease, often in the gastrointestinal tract (gut). Colonization is also a known risk factor for infection.

As a result, the disposal of waste from an infected or colonized person or animal can become a source of resistant bacteria in the environment. Once resistant microbes are in the environment, there is the potential to spread, colonize, or cause infections in other people or animals. Resistance in bacteria known to cause human infections is of particular concern, as well as bacteria carrying mobile resistance determinants (e.g., resistance genes on plasmids) that confer resistance to medically important antimicrobials.

In addition to resistance, this waste can also be a source of medically important antimicrobials in the environment. If these antimicrobials retain their activity in the environment, they can apply selective pressure on the microbial population and amplify resistant bacteria.

The connection between human and animal waste in the environment and its impact on human health is not well understood and warrants additional study to address knowledge gaps. This work should be performed using methods and sampling strategies that determine the type of resistance, the concentration of resistant bacteria, the source of contamination (i.e., attribution), and how much resistance has spread (or disseminated).

The response to environmental contamination of AMR could include prevention strategies (e.g., pre-treating sewage from elevated sources, like hospitals, before release) and removal strategies (e.g., wastewater treatment processes). Suitable research methods and data collection should also measure the impact of interventions that are used to prevent or remove this environmental contamination. It is important to understand the effectiveness of existing practices for waste management and water processing, as well as investigating novel methods and strategies.

Scientific Issues

A. To what extent are human waste or animal waste contaminating the environment with antibiotic-resistant pathogens, specifically from hospitals, human sewage, animal farms, and aquaculture? What strategies should be used to track antimicrobial-resistant pathogens or antimicrobial contamination from each source?

Hospitals

There are several issues to consider regarding the risk of environmental contamination from hospitals. For example, some of the most resistant infections occur in inpatients, who stay in a hospital while undergoing treatment and are commonly administered antimicrobials. Basic infection control practices and sanitation practices are essential to prevent transmission of antimicrobial-resistant microbes from patient to patient and from patient to healthcare workers. Additionally, antimicrobials and pathogenic antimicrobial-resistant microbes from patient urine and fecal matter are typically released into a facility's wastewater collection system. Untreated or partially treated wastewater effluents are a source of antimicrobials and antimicrobial-resistant microbes in the environment. Robust wastewater treatment either at the facility or downstream (in the sewage system) of the facility is needed to prevent unnecessary exposure to people or animals. Inside the facility, antimicrobial-resistant microbes can persist and grow within the healthcare facility plumbing system, such as sink drains, taps, and other sources of water. This reservoir of AMR can contribute to transmission of resistance within hospitals, and may contribute to the load of AMR in hospital wastewater effluent.

Drivers of Antimicrobial-resistant Bacteria within Healthcare Facilities

Antimicrobial use and the spread of antimicrobial-resistant microbes are drivers of resistance in healthcare facilities. Antimicrobial use selects for and amplifies antimicrobial-resistant microbes. For example, using antimicrobials for inpatients is common. In Europe, 20-30% of acute care inpatients received antimicrobials,[ADDIN EN.CITE
<EndNote><Cite><Author>Ansari</Author><Year>2009</Year><RecNum>57</RecNum><DisplayText><style face="superscript">[1]</style></DisplayText><record><rec-number>57</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971961">57</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Ansari, Faranak</author><author>Erntell, Mats</author><author>Goossens, Herman</author><author>Davey, Peter</author><author>Esac Ii

Hospital Care Study Group</author></authors></contributors><titles><title>The European Surveillance of Antimicrobial Consumption (ESAC) Point-Prevalence Survey of Antibacterial Use in 20 European Hospitals in 2006</title><secondary-title>Clinical Infectious Diseases</secondary-title></titles><periodical><full-title>Clinical Infectious Diseases</full-title></periodical><pages>1496-1504</pages><volume>49</volume><number>10</number><dates><year>2009</year></dates><isbn>1058-4838</isbn><urls><related-urls><url>http://dx.doi.org/10.1086/644617</url></related-urls></urls><electronic-resource-num>10.1086/644617</electronic-resource-num></record></Cite></EndNote>] and 1 in 2 patients received an antimicrobial for at least 1 day in U.S. hospitals.[ADDIN EN.CITE <EndNote><Cite><Author>CDC</Author><Year>2017</Year><RecNum>608</RecNum><DisplayText><style face="superscript">[2]</style></DisplayText><record><rec-number>608</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1531739394">608</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>CDC</author></authors></contributors><titles><title>Antibiotic Use in the United States, 2017: Progress and Opportunities</title></titles><dates><year>2017</year></dates><pub-location>Atlanta, GA</pub-location><publisher>US Department of Healtn and Human Services, CDC</publisher><urls></urls></record></Cite></EndNote>] Antimicrobial-resistant microbes can be transmitted from person to person or from the hospital environment (e.g., equipment, sinks) to people. Both factors contribute to a population of patients who are at an increased risk of being infected or colonized with antimicrobial-resistant microbes, which then contributes to AMR and potentially active antimicrobials released into wastewater through the healthcare facility plumbing system.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

As mentioned, the disposal of human waste containing antimicrobial-resistant microbes can also be a potential threat to people inside the hospital. For example, a study found carbapenem-resistant Enterobacteriaceae (CRE) in the trap of hospital room sinks, and it grew in the direction of the sink strainer. Splatter from the strainer exposed new patients to CRE.[ADDIN EN.CITE <EndNote><Cite><Author>Kotay</Author><Year>2017</Year><RecNum>611</RecNum><DisplayText><style face="superscript">[4]</style></DisplayText><record><rec-number>611</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1533747432">611</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kotay, Shireen</author><author>Chai, Weidong</author><author>Guilford, William</author><author>Barry, Katie</author><author>Mathers, Amy J.</author></authors></contributors><titles><title>Spread from the Sink to the Patient: In Situ Study Using Green Fluorescent Protein (GFP)-Expressing Escherichia coli To Model Bacterial Dispersion from Hand-Washing Sink-Trap Reservoirs</title><secondary-title>Applied and Environmental Microbiology</secondary-title></titles><periodical><full-title>Applied and Environmental Microbiology</full-title></periodical><volume>83</volume><number>8</number><dates><year>2017</year><pub-dates><date>April 15, 2017</date></pub-dates></dates><urls><related-urls><url>http://aem.asm.org/content/83/8/e03327-16.abstract</url></related-urls></urls><electronic-resource-num>10.1128/aem.03327-16</electronic-resource-num></record></Cite></EndNote>] These findings represent a new infection control challenge for healthcare facilities. It is important to understand how much plumbing contributes to antimicrobial-resistant infections in hospitals and identify effective mitigation strategies.

Characteristics of Healthcare Facility Wastewaters

Hospital wastewater can be a source of antimicrobial-resistant microbes. Current regulations for hospital waste disposal were developed before the risk of environmental contamination related to antimicrobial-resistant microbes and antimicrobials were considered. The extent of antimicrobial-resistant microbes released in wastewater from a healthcare facility depends on the type of healthcare facility, including the size, management, and location. There are also wide differences in how healthcare facilities handle and dispose of wastewater. For example, some countries, require healthcare facilities to have their own wastewater treatment plants, but these treatment systems are often not well maintained. In other countries, community treatment plants use a three-step process for treating all wastewater including that from healthcare facilities.

Common multi-drug resistant bacteria recovered from untreated hospital wastewater include extended-spectrum β-lactamase (ESBL)-producing or carbapenemase-producing Enterobacteriaceae, vancomycin-resistant enterococci (VRE), and *Pseudomonas aeruginosa*.[ADDIN EN.CITE <EndNote><Cite><Author>Hocquet</Author><Year>2016</Year><RecNum>58</RecNum><DisplayText><style face="superscript">[5]</style></DisplayText><record><rec-number>58</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972039">58</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hocquet, D.</author><author>Muller,

A.</author><author>Bertrand, X.</author></authors></contributors><titles><title>What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems</title><secondary-title>Journal of Hospital Infection</secondary-title></titles><periodical><full-title>Journal of Hospital Infection</full-title></periodical><pages>395-402</pages><volume>93</volume><number>4</number><keywords><keyword>Extended-spectrum β-lactamase</keyword><keyword>Multidrug resistance</keyword><keyword>Vancomycin-resistant enterococcus</keyword><keyword>Wastewater</keyword><keyword>Wastewater treatment plants</keyword></keywords><dates><year>2016</year><pub-dates><date>2016/08/01/</date></pub-dates></dates><isbn>0195-6701</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0195670116000645</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.jhin.2016.01.010</electronic-resource-num></record></Cite></EndNote>] There is evidence that the concentrations of many bacteria are similar in urban and hospital wastewater, but the proportion of resistant enteric (gut) bacteria are often higher in hospital effluent. This was demonstrated for VRE, which were significantly more prevalent in hospital effluent when compared to community effluent.[ADDIN EN.CITE ADDIN EN.CITE.DATA] In Bangladesh, the prevalence of NDM-1-positive bacteria (i.e., CRE) in wastewater samples close to hospitals was significantly higher than in community wastewater samples from the same city (71% vs 12.1%).[ADDIN EN.CITE
<EndNote><Cite><Author>Islam</Author><Year>2017</Year><RecNum>137</RecNum><DisplayText><style face="superscript">[8]</style></DisplayText><record><rec-number>137</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972455">137</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Islam, Mohammad Aminul</author><author>Islam, Moydul</author><author>Hasan, Rashedul</author><author>Hossain, M. Iqbal</author><author>Nabi, Ashikun</author><author>Rahman, Mahdia</author><author>Goessens, Wil H. F.</author><author>Endtz, Hubert P.</author><author>Boehm, Alexandria B.</author><author>Faruque, Shah M.</author></authors></contributors><titles><title>Environmental Spread of New Delhi Metallo-β-Lactamase-1-Producing Multidrug-Resistant Bacteria in Dhaka, Bangladesh</title><secondary-title>Applied and Environmental Microbiology</secondary-title></titles><periodical><full-title>Applied and Environmental Microbiology</full-title></periodical><volume>83</volume><number>15</number><dates><year>2017</year><pub-dates><date>August 1, 2017</date></pub-dates></dates><urls><related-

urls><url>http://aem.asm.org/content/83/15/e00793-17.abstract</url></related-urls></urls><electronic-resource-num>10.1128/aem.00793-17</electronic-resource-num></record></Cite></EndNote>]

In some cases, antimicrobial residue concentrations in hospital effluent corresponded with the most common antimicrobials used in hospitals. For example, in India, there was a correlation between using the antimicrobial ciprofloxacin and concentrations of ciprofloxacin in hospital effluent,[ADDIN EN.CITE <EndNote><Cite><Author>Diwan</Author><Year>2010</Year><RecNum>145</RecNum><DisplayText><style face="superscript">[9]</style></DisplayText><record><rec-number>145</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523973183">145</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Diwan, Vishal</author><author>Tamhankar, Ashok J.</author><author>Khandal, Rakesh K.</author><author>Sen, Shanta</author><author>Aggarwal, Manjeet</author><author>Marothi, Yogyata</author><author>Iyer, Rama V.</author><author>Sundblad-Tonderski, Karin</author><author>Stålsby- Lundborg, Cecilia</author></authors></contributors><titles><title>Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India</title><secondary-title>BMC Public Health</secondary-title></titles><periodical><full-title>BMC Public Health</full-title></periodical><pages>414</pages><volume>10</volume><number>1</number><dates><year>2010</year><pub-dates><date>July 13</date></pub-dates></dates><isbn>1471-2458</isbn><label>Diwan2010</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1186/1471-2458-10-414</url></related-urls></urls><electronic-resource-num>10.1186/1471-2458-10-414</electronic-resource-num></record></Cite></EndNote>] but the effect of these antimicrobials on *Escherichia coli* (*E. coli*) isolates recovered from environmental waters samples was not clear. Furthermore, there is growing evidence that pathogenic antimicrobial-resistant bacteria from hospitals tend to carry more antimicrobial-resistant genes (ARGs) per cell.[ADDIN EN.CITE <EndNote><Cite><Author>Devarajan</Author><Year>2016</Year><RecNum>607</RecNum><DisplayText><style face="superscript">[10]</style></DisplayText><record><rec-number>607</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1531229825">607</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Devarajan, Naresh</author><author>Laffite, Amandine</author><author>Mulaji, Crispin Kyela</author><author>Otamonga, Jean-Paul</author><author>Mpiana, Pius Tshimankinda</author><author>Mubedi, Josué

Ilunga</author><author>Prabakar, Kandasamy</author><author>Ibelings, Bastiaan Willem</author><author>Poté, John</author></authors></contributors><titles><title>Occurrence of Antibiotic Resistance Genes and Bacterial Markers in a Tropical River Receiving Hospital and Urban Wastewaters</title><secondary-title>PLoS ONE</secondary-title></titles><periodical><full-title>PLoS One</full-title></periodical><pages>e0149211</pages><volume>11</volume><number>2</number><dates><year>2016</year><pub-dates><date>02/24
10/14/received
01/28/accepted</date></pub-dates></dates><pub-location>San Francisco, CA USA</pub-location><publisher>Public Library of Science</publisher><isbn>1932-6203</isbn><accession-num>PMC4766091</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4766091/</url></related-urls></urls><electronic-resource-num>10.1371/journal.pone.0149211</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] Absolute levels of pathogenic antimicrobial-resistant bacteria and genes are typically more than 10 times higher in hospital waste compared to community wastes.[ADDIN EN.CITE ADDIN EN.CITE.DATA] For example, a recent Indian study showed carbapenem-resistant enteric bacteria were 100 to 1,000 times greater in hospital wastewaters than community wastewaters and related antimicrobial-resistant genes were almost 100,000 times higher from hospital sources.[ADDIN EN.CITE <EndNote><Cite><Author>Lamba</Author><Year>2018</Year><RecNum>600</RecNum><DisplayText><style face="superscript">[13]</style></DisplayText><record><rec-number>600</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1531143662">600</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lamba, M., Graham, DW, Sreekrishnan, TR, Ahammad</author></authors></contributors><titles><title>Carbapenem resistance exposures via wastewaters across New Delhi</title><secondary-title>Environment International</secondary-title></titles><periodical><full-title>Environment International</full-title></periodical><dates><year>2018</year><pub-dates><date>in press</date></pub-dates></dates><urls></urls></record></Cite></EndNote>] Of particular concern are Enterobacteriaceae that can carry multiple ARGs on plasmids, which can move from bacteria to bacteria through horizontal gene transfer.[ADDIN EN.CITE <EndNote><Cite><Author>Quintela-Baluja</Author><Year>2018</Year><RecNum>142</RecNum><DisplayText><style face="superscript">[14]</style></DisplayText><record><rec-number>142</rec-number><foreign-

keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972981">142</key></foreign-keys><ref-type name="Thesis">32</ref-type><contributors><authors><author>Quintela-Baluja, M</author></authors></contributors><titles><title>Urban water cycle and antibiotic resistance genes dissemination</title></titles><volume>Doctor of Philosophy</volume><dates><year>2018</year></dates><publisher>Newcastle University</publisher><urls></urls></record></Cite></EndNote>]

However, this information is based on limited studies and more knowledge is needed to determine whether source-treatment of healthcare facility wastes is the best intervention or if other interventions should be considered. There is no absolute proof that multi-drug resistant pathogens in hospital wastes pose a greater risk to human health than comparable organisms from the community. Evidence does suggest that enteric bacteria from hospitals are more likely to be resistant[ADDIN EN.CITE <EndNote><Cite><Author>Quintela-Baluja</Author><Year>2018</Year><RecNum>142</RecNum><DisplayText><style face="superscript">[14]</style></DisplayText><record><rec-number>142</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972981">142</key></foreign-keys><ref-type name="Thesis">32</ref-type><contributors><authors><author>Quintela-Baluja, M</author></authors></contributors><titles><title>Urban water cycle and antibiotic resistance genes dissemination</title></titles><volume>Doctor of Philosophy</volume><dates><year>2018</year></dates><publisher>Newcastle University</publisher><urls></urls></record></Cite></EndNote>] and these bacteria are able to share this resistance with other bacteria through horizontal gene transfer, but work is needed to determine the specific risk to human health from hospital wastewater.

Antimicrobial-resistant bacteria detected in wastewater can correlate to the antimicrobial-resistant bacteria causing infections within the facility,[ADDIN EN.CITE <EndNote><Cite><Author>Varela</Author><Year>2014</Year><RecNum>138</RecNum><DisplayText><style face="superscript">[15]</style></DisplayText><record><rec-number>138</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972485">138</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Varela, Ana Rita</author><author>André, Sandra</author><author>Nunes, Olga C.</author><author>Manaia, Célia M.</author></authors></contributors><titles><title>Insights into the relationship between antimicrobial residues and bacterial populations in a hospital-urban wastewater treatment plant system</title><secondary-title>Water Research</secondary-title></titles><periodical><full-title>Water Research</full-title></periodical><pages>327-336</pages><volume>54</volume><keywords><keyword>Wastewater</keyword><keyword>Hospital effluents</keyword><keyword>Antimicrobial residues</keyword><keyword>Antibiotic resistance</keyword><keyword>Bacterial communities</keyword></keywords><dates><year>2014</year><pub-dates><date>2014/05/01/</date></pub-dates></dates><isbn>0043-1354</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0043135414001134</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.watres.2014.02.003</electronic-resource-num></record></Cite></EndNote>] but that is not always the case. The fact that hospital effluent almost always mixes with wastewater from the community makes it difficult to determine the original source of specific ARGs or resistant bacteria that are received at community WWTPs. This is particularly challenging in locations where there is a comparatively high prevalence of antimicrobial-resistant bacteria in the wider human or animal population, or the natural environment.[ADDIN EN.CITE <EndNote><Cite><Author>Graham</Author><Year>2014</Year><RecNum>136</RecNum><DisplayText><style face="superscript">[16]</style></DisplayText><record><rec-number>136</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972425">136</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Graham, David W.</author><author>Collignon, Peter</author><author>Davies, Julian</author><author>Larsson, D. G. Joakim</author><author>Snape, Jason</author></authors></contributors><titles><title>Underappreciated Role of Regionally Poor Water Quality on Globally Increasing Antibiotic Resistance</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>11746-11747</pages><volume>48</volume><number>20</number><dates><year>2014</year><pub-dates><date>2014/10/21</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/es504206x</url></related-urls></urls><electronic-resource-num>10.1021/es504206x</electronic-resource-num></record></Cite></EndNote>] Clearly defining the root source of antimicrobial-resistant bacteria detected in a given wastewater influent is difficult and is a knowledge gap in understanding which mitigation measures will be most effective.

Similarly, levels of antimicrobials detected in wastewater do not always correlate with antimicrobial use in a healthcare facility. This is partly because degradation of antimicrobials and survival of bacteria in the environment depends on several factors. For example, antimicrobial half-lives range widely from minutes to tens of days,[ADDIN EN.CITE <EndNote><Cite><Author>Homem</Author><Year>2011</Year><RecNum>139</RecNum><DisplayText><style face="superscript">[17]</style></DisplayText><record><rec-number>139</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972673">139</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Homem, Vera</author><author>Santos, Lúcia</author></authors></contributors><titles><title>Degradation and removal methods of antibiotics from aqueous matrices – A review</title><secondary-title>Journal of Environmental Management</secondary-title></titles><periodical><full-title>Journal of Environmental Management</full-title></periodical><pages>2304-2347</pages><volume>92</volume><number>10</number><keywords><keyword>Antibiotics</keyword><keyword>Emergent pollutants</keyword><keyword>Degradation/removal processes</keyword></keywords><dates><year>2011</year><pub-dates><date>2011/10/01/</date></pub-dates></dates><isbn>0301-4797</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0301479711001782</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.jenvman.2011.05.023</electronic-resource-num></record></Cite></EndNote>] and survival rates of resistant bacteria are also geographically-dependent and highly variable. The relationship of both antimicrobials and antimicrobial-resistant microbes in wastewater also depends on location because there are different environmental temperatures and different resistant colonization rates across the globe.[ADDIN EN.CITE <EndNote><Cite><Author>Lamba</Author><Year>2018</Year><RecNum>600</RecNum><DisplayText><style face="superscript">[13]</style></DisplayText><record><rec-number>600</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1531143662">600</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lamba, M., Graham, DW, Sreekrishnan, TR, Ahammad</author></authors></contributors><titles><title>Carbapenem resistance exposures via wastewaters across New Delhi</title><secondary-title>Environment International</secondary-

title></titles><periodical><full-title>Environment International</full-title></periodical><dates><year>2018</year><pub-dates><date>in press</date></pub-dates></dates><urls></urls></record></Cite></EndNote>]

Mixing Healthcare Facility Wastewaters and Community Wastewaters

The point at which healthcare facility wastewater is mixed with wastewaters from the wider community seems to be an important factor related to the type of antimicrobial-resistant microbes that move further downstream in sewer systems, ultimately to WWTPs.[ADDIN EN.CITE <EndNote><Cite><Author>Tyson</Author><Year>2015</Year><RecNum>538</RecNum><DisplayText><style face="superscript">[18]</style></DisplayText><record><rec-number>538</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529500054">538</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Tyson, Gregory H.</author><author>McDermott, Patrick F.</author><author>Li, Cong</author><author>Chen, Yuansha</author><author>Tadesse, Daniel A.</author><author>Mukherjee, Sampa</author><author>Bodeis-Jones, Sonya</author><author>Kabera, Claudine</author><author>Gaines, Stuart A.</author><author>Loneragan, Guy H.</author><author>Edrington, Tom S.</author><author>Torrence, Mary</author><author>Harhay, Dayna M.</author><author>Zhao, Shaohua</author></authors></contributors><titles><title>WGS accurately predicts antimicrobial resistance in Escherichia coli</title><secondary-title>Journal of Antimicrobial Chemotherapy</secondary-title></titles><periodical><full-title>Journal of Antimicrobial Chemotherapy</full-title></periodical><pages>2763-2769</pages><volume>70</volume><number>10</number><dates><year>2015</year></dates><isbn>0305-7453</isbn><urls><related-urls><url>http://dx.doi.org/10.1093/jac/dkv186</url></related-urls></urls><electronic-resource-num>10.1093/jac/dkv186</electronic-resource-num></record></Cite></EndNote>] Bacteria are known to accelerate horizontal gene transfer when stressed, so changes in their local habitat influence the rates at which they exchange genes and evolve, including sharing ARGs. Factors that affect horizontal gene transfer at the mixing point in sewers include temperature differences, the presence of co-selective metals and biocides, and basic differences between bacteria found in healthcare, community, and environmental settings.

However, there is debate about the relative importance and differences between hospital and community waste streams.[ADDIN EN.CITE

<EndNote><Cite><Author>Wang</Author><Year>2018</Year><RecNum>144</RecNum><DisplayText><style face="superscript">[19]</style></DisplayText><record><rec-number>144</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523973154">144</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wang, Qiang</author><author>Wang, Panliang</author><author>Yang, Qingxiang</author></authors></contributors><titles><title>Occurrence and diversity of antibiotic resistance in untreated hospital wastewater</title><secondary-title>Science of The Total Environment</secondary-title></titles><periodical><full-title>Science of The Total Environment</full-title></periodical><pages>990-999</pages><volume>621</volume><keywords><keyword>Antibiotic-resistant bacteria</keyword><keyword>Antibiotic-resistance gene</keyword><keyword>Mobile genetic element</keyword><keyword>Gene cassette</keyword><keyword>Hospital wastewater</keyword></keywords><dates><year>2018</year><pub-dates><date>2018/04/15/</date></pub-dates></dates><isbn>0048-9697</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0048969717328383</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.scitotenv.2017.10.128</electronic-resource-num></record></Cite></EndNote>] Early findings suggest that healthcare-related bacteria have a greater potential for horizontal gene transfer and might have selective advantages that enhance their survival in wastewater treatment. More data are needed to confirm this observation. A key knowledge gap is whether microbial isolates from hospital wastewaters pose a greater risk to human health than microbes found in community wastewaters. Recent data suggests they are different and new analytical methods are being developed to clarify this key question.[ADDIN EN.CITE <EndNote><Cite><Author>Proia</Author><Year>2018</Year><RecNum>596</RecNum><DisplayText><style face="superscript">[11]</style></DisplayText><record><rec-number>596</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1530537793">596</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Proia, Lorenzo</author><author>Anzil, Adriana</author><author>Borrego, Carles</author><author>Farrè, Marinella</author><author>Llorca, Marta</author><author>Sanchis, Josep</author><author>Bogaerts, Pierre</author><author>Balcázar, Jose Luis</author><author>Servais, Pierre</author></authors></contributors><titles><title>Occurrence and persistence of carbapenemases genes in hospital and wastewater treatment plants and propagation in the receiving river</title><secondary-title>Journal of Hazardous Materials</secondary-

title></titles><periodical><full-title>Journal of Hazardous Materials</full-title></periodical><pages>33-43</pages><volume>358</volume><keywords><keyword>Carbapenemases genes</keyword><keyword>Urban River</keyword><keyword>ARGs</keyword><keyword>Wastewaters</keyword><keyword>Hospital</keyword></keywords><dates><year>2018</year><pub-dates><date>9/15/</date></pub-dates></dates><isbn>0304-3894</isbn><urls><related-urls><url>https://www.sciencedirect.com/science/article/pii/S0304389418304989</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.jhazmat.2018.06.058</electronic-resource-num></record></Cite></EndNote>] Currently, this gap in knowledge makes it difficult to determine the specific risk of healthcare facility wastewater in a conclusive way.

Human Sewage

Human sewage contains pathogenic and commensal (non-disease-causing) enteric microbes carrying ARGs. Many potentially disease-causing bacteria, including *E. coli, Klebsiella pneumoniae*, and *Acinetobacter baumannii*, colonize in the gastrointestinal tract of animals and humans and, when resistant, contribute to AMR in human sewage.[ADDIN EN.CITE <EndNote><Cite><Author>Sobsey MD</Author><Year>2014</Year><RecNum>570</RecNum><DisplayText><style face="superscript">[20]</style></DisplayText><record><rec-number>570</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529513991">570</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Sobsey MD, Abebe L, Andremont A, Ashbolt NJ, Husman AM de R, Gin KY-H, Hunter PR, Meschke JS, Vilchez S. </author></authors></contributors><titles><title>Briefing Notes - Antimicrobial Resistance: An Emerging Water, Sanitation and Hygiene Issue</title></titles><dates><year>2014</year></dates><publisher>World Health Organization</publisher><urls></urls></record></Cite></EndNote>] For example, *E. coli* naturally occurs in humans, animals, and the environment, making it a concern for community-associated AMR. It is also associated with resistant mechanisms that move easily between bacteria, like ESBLs and carbapenemases.[ADDIN EN.CITE <EndNote><Cite><Author>Sobsey MD</Author><Year>2014</Year><RecNum>570</RecNum><DisplayText><style face="superscript">[20]</style></DisplayText><record><rec-number>570</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"

timestamp="1529513991">570</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Sobsey MD, Abebe L, Andremont A, Ashbolt NJ, Husman AM de R, Gin KY-H, Hunter PR, Meschke JS, Vilchez S. </author></authors></contributors><titles><title>Briefing Notes - Antimicrobial Resistance: An Emerging Water, Sanitation and Hygiene Issue</title></titles><dates><year>2014</year></dates><publisher>World Health Organization</publisher><urls></urls></record></Cite></EndNote>] Globally, an estimated 14% of healthy humans are colonized by ESBL-producing Enterobacteriaceae, with prevalence rates as high as 22% in Southeast Asia and Africa.[ADDIN EN.CITE <EndNote><Cite><Author>Karanika</Author><Year>2016</Year><RecNum>147</RecNum><DisplayText><style face="superscript">[21]</style></DisplayText><record><rec-number>147</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523977247">147</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Karanika, Styliani</author><author>Karantanos, Theodoros</author><author>Arvanitis, Marios</author><author>Grigoras, Christos</author><author>Mylonakis, Eleftherios</author></authors></contributors><titles><title>Fecal Colonization With Extended-spectrum Beta-lactamase–Producing Enterobacteriaceae and Risk Factors Among Healthy Individuals: A Systematic Review and Metaanalysis</title><secondary-title>Clinical Infectious Diseases</secondary-title></titles><periodical><full-title>Clinical Infectious Diseases</full-title></periodical><pages>310-318</pages><volume>63</volume><number>3</number><dates><year>2016</year></dates><isbn>1058-4838</isbn><urls><related-urls><url>http://dx.doi.org/10.1093/cid/ciw283</url></related-urls></urls><electronic-resource-num>10.1093/cid/ciw283</electronic-resource-num></record></Cite></EndNote>] When these and other bacteria are released into sewage, wastewater, and, subsequently, onto land or surface waters, it contributes to the environmental resistome (the collection of all the antimicrobial resistance genes and their precursors in both pathogenic and non-pathogenic bacteria).

WWTPs are essential for reducing fecal microbes, including resistant microbes from wastewater, but when levels of antimicrobial-resistant microbes are high, traditional systems may not be sufficient. Antimicrobial-resistant microbes can persist even in advanced WWTPs and remain at detectable levels in surface waters receiving the discharge.[ADDIN EN.CITE <EndNote><Cite><Author>LaPara</Author><Year>2011</Year><RecNum>150</RecNum><DisplayText>

<style face="superscript">[22]</style></DisplayText><record><rec-number>150</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523983203">150</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>LaPara, Timothy M.</author><author>Burch, Tucker R.</author><author>McNamara, Patrick J.</author><author>Tan, David T.</author><author>Yan, Mi</author><author>Eichmiller, Jessica J.</author></authors></contributors><titles><title>Tertiary-Treated Municipal Wastewater is a Significant Point Source of Antibiotic Resistance Genes into Duluth-Superior Harbor</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>9543-9549</pages><volume>45</volume><number>22</number><dates><year>2011</year><pub-dates><date>2011/11/15</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/es202775r</url></related-urls></urls><electronic-resource-num>10.1021/es202775r</electronic-resource-num></record></Cite></EndNote>] While sewage effluent might be diluted when it is released into the environment through rivers, estuaries, or coastal waters, it still interacts with the microbes in the natural environment.[ADDIN EN.CITE <EndNote><Cite><Author>Singer</Author><Year>2016</Year><RecNum>149</RecNum><DisplayText><style face="superscript">[23]</style></DisplayText><record><rec-number>149</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523983175">149</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Singer, Andrew C.</author><author>Shaw, Helen</author><author>Rhodes, Vicki</author><author>Hart, Alwyn</author></authors></contributors><titles><title>Review of Antimicrobial Resistance in the Environment and Its Relevance to Environmental Regulators</title><secondary-title>Frontiers in Microbiology</secondary-title></titles><periodical><full-title>Frontiers in Microbiology</full-title></periodical><pages>1728</pages><volume>7</volume><dates><year>2016</year><pub-dates><date>11/01
07/12/received
10/17/accepted</date></pub-dates></dates><publisher>Frontiers Media S.A.</publisher><isbn>1664-302X</isbn><accession-num>PMC5088501</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5088501/</url></related-urls></urls><electronic-resource-num>10.3389/fmicb.2016.01728</electronic-resource-num><remote-

database-name>PMC</remote-database-name></record></Cite></EndNote>] Untreated human waste might also be inadvertently released directly into water bodies (e.g., overflow of combined sewers). There are recent studies in the U.S. that have found a surprising amount of human waste contamination in the environment from sources like septic systems in rural areas and storm water outfalls in urban areas.[ADDIN EN.CITE ADDIN EN.CITE.DATA] These findings could indicate poorly maintained septic systems, insufficient wastewater processing capacity, or failing infrastructure.

A lack of sanitation infrastructure in many urban centers around the world means that only a portion of human sewage is appropriately treated (e.g., 56% in Delhi, India; 55% in Kumasi City, Ghana). In Dhaka, Bangladesh, only 1% of human waste is effectively treated, and 70% is discharged directly into the environment.[ADDIN EN.CITE
<EndNote><Cite><Author>Peal</Author><Year>2015</Year><RecNum>158</RecNum><DisplayText><style face="superscript">[26]</style></DisplayText><record><rec-number>158</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523983746">158</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Peal, A., Evans, B., Blackett, I., Hawkins, P., Heymans, P</author></authors></contributors><titles><title>A review of fecal sludge management in 12 cities. World Bank - Water and Sanitation Program</title></titles><dates><year>2015</year></dates><urls><related-urls><url>http://www.susana.org/_resources/documents/default/3-2212-7-1435304068.pdf</url></related-urls></urls></record></Cite></EndNote>]

Within treatment plants, microbial communities might be further exposed to antimicrobials, although at very low concentrations. For example, 56 antimicrobials belonging to six different classes were detected at nanogram-per-liter (ng/L) to microgram-per-liter (μg/L) levels in the influent and effluent of WWTPs in East Asia, North America, Europe, and Australia, corresponding closely with the most commonly prescribed antimicrobials for human use.[ADDIN EN.CITE
<EndNote><Cite><Author>Zhang</Author><Year>2011</Year><RecNum>152</RecNum><DisplayText><style face="superscript">[27]</style></DisplayText><record><rec-number>152</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523983285">152</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zhang, Tong</author><author>Li, Bing</author></authors></contributors><titles><title>Occurrence, Transformation, and Fate of

Antibiotics in Municipal Wastewater Treatment Plants</title><secondary-title>Critical Reviews in Environmental Science and Technology</secondary-title></titles><periodical><full-title>Critical Reviews in Environmental Science and Technology</full-title></periodical><pages>951-998</pages><volume>41</volume><number>11</number><dates><year>2011</year><pub-dates><date>2011/04/26</date></pub-dates></dates><publisher>Taylor & Francis</publisher><isbn>1064-3389</isbn><urls><related-urls><url>https://doi.org/10.1080/10643380903392692</url></related-urls></urls><electronic-resource-num>10.1080/10643380903392692</electronic-resource-num></record></Cite></EndNote>] Even these low concentrations can alter microbial communities and select for resistance in microbes (see section entitled “Antimicrobial Manufacturing Waste” for more information about the selective pressure of antimicrobials in the environment).[ADDIN EN.CITE ADDIN EN.CITE.DATA] The concentrations of antimicrobial residues have not been assessed in many low- and middle-income countries, and therefore the potential risk to human health is unknown.

Additionally, there are concerns around using treated sewage sludge (biosolids) on agricultural land. When properly treated and processed, sewage sludge becomes biosolids, which are nutrient-rich organic materials largely composed of human waste produced from wastewater treatment facilities. Biosolids can be recycled and applied as fertilizer to improve and maintain productive soils and stimulate plant growth.[ADDIN EN.CITE <EndNote><Cite><RecNum>591</RecNum><DisplayText><style face="superscript">[31]</style></DisplayText><record><rec-number>591</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529929409">591</key></foreign-keys><ref-type name="Web Page">12</ref-type><contributors></contributors><titles><title>Basic Information about Biosolids</title></titles><volume>2018</volume><number>June 25</number><dates></dates><publisher>U.S. Environmental Protection Agency</publisher><urls><related-urls><url>https://www.epa.gov/biosolids/basic-information-about-biosolids</url></related-urls></urls></record></Cite></EndNote>] In Europe, a study found trace levels of antimicrobials and evidence of resistant bacteria like ESBL-producers in treated sewage sludge, demonstrating that treatment without some sort of disinfection might not be enough to remove these contaminants.[ADDIN EN.CITE <EndNote><Cite><Author>Wellington</Author><Year>2013</Year><RecNum>151</RecNum><DisplayText><style face="superscript">[32]</style></DisplayText><record><rec-number>151</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"

timestamp="1523983233">151</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wellington, Elizabeth M. H.</author><author>Boxall, Alistair B. A.</author><author>Cross, Paul</author><author>Feil, Edward J.</author><author>Gaze, William H.</author><author>Hawkey, Peter M.</author><author>Johnson-Rollings, Ashley S.</author><author>Jones, Davey L.</author><author>Lee, Nicholas M.</author><author>Otten, Wilfred</author><author>Thomas, Christopher M.</author><author>Williams, A. Prysor</author></authors></contributors><titles><title>The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria</title><secondary-title>The Lancet Infectious Diseases</secondary-title></titles><periodical><full-title>The Lancet Infectious Diseases</full-title></periodical><pages>155-165</pages><volume>13</volume><number>2</number><dates><year>2013</year><pub-dates><date>2013/02/01/</date></pub-dates></dates><isbn>1473-3099</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S1473309912703171</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/S1473-3099(12)70317-1</electronic-resource-num></record></Cite></EndNote>] Currently, there is limited understanding of the environmental consequences from these trace chemical and biological contaminants. However, recent studies suggest human exposure and environmental transmission does occur.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Waste from Animal Farms

Wastes Generated or Used in Agriculture as a Source of AMR

Antimicrobial-resistant bacteria, including bacteria resistant to multiple classes of antimicrobials, are found in animal manures, from food-producing animal farms. Resistance occurs from the selective pressure of antimicrobials and other agents with co-selection potential (e.g., metals) that are commonly applied in food animal production systems.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Antimicrobial-resistant bacteria can also be introduced via biosolids used to fertilize agricultural land.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Data from the U.S. National Antimicrobial Resistance Monitoring System (NARMS)—a culture-based nationwide surveillance effort focused on resistance in humans, fresh retail meat products, and food animals—show that resistance in bacteria causing foodborne illness has declined or has held steady for more than a decade.[ADDIN EN.CITE

<EndNote><Cite><Author>FDA</Author><Year>2015</Year><RecNum>175</RecNum><DisplayText><st

yle face="superscript">[46]</style></DisplayText><record><rec-number>175</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523991558">175</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><authors><author>FDA</author></authors></contributors><titles><title>National Antimicrobial Resistance Monitoring System – Enteric. Bacteria (NARMS)</title></titles><dates><year>2015</year></dates><pub-location>Rockville, MD: U.S.</pub-location><urls></urls><custom1>FDA</custom1></record></Cite></EndNote>] However, NARMS does not track antimicrobial resistance in commensal (i.e., non-pathogenic) bacteria so the potential contribution of resistance in these bacteria to the farm resistome is unknown.

Bacteria from food-producing animals carry antimicrobial-resistant mechanisms on mobile genetic elements, such as plasmids. This increases the risk of resistance transfer from animal bacteria to bacteria that commonly colonize or infect humans. For example, plasmids carrying a cephalosporinase called *bla*CMY-2 are widespread in *Salmonella* and *Enterobacteriaceae* in North American cattle.[ADDIN EN.CITE <EndNote><Cite><Author>Mollenkopf</Author><Year>2017</Year><RecNum>190</RecNum><DisplayText><style face="superscript">[47]</style></DisplayText><record><rec-number>190</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523992197">190</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mollenkopf, D. F.</author><author>Mathys, D. A.</author><author>Dargatz, D. A.</author><author>Erdman, M. M.</author><author>Habing, G. G.</author><author>Daniels, J. B.</author><author>Wittum, T. E.</author></authors></contributors><titles><title>Genotypic and epidemiologic characterization of extended-spectrum cephalosporin resistant Salmonella enterica from US beef feedlots</title><secondary-title>Preventive Veterinary Medicine</secondary-title></titles><periodical><full-title>Preventive Veterinary Medicine</full-title></periodical><pages>143-149</pages><volume>146</volume><keywords><keyword>National animal health monitoring system</keyword><keyword>Extended-spectrum cephalosporin resistance</keyword><keyword>Beef cattle</keyword></keywords><dates><year>2017</year><pub-dates><date>2017/10/01/</date></pub-dates></dates><isbn>0167-5877</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0167587717301782</url></related-

urls></urls><electronic-resource-num>https://doi.org/10.1016/j.prevetmed.2017.08.006</electronic-resource-num></record></Cite></EndNote>].

Animal manure can carry both antimicrobials and resistant bacteria. Food animals generally urinate and defecate antimicrobials without any degradation. The amount of time the antimicrobials stay in the environment depends on various factors. The presence of antimicrobials can increase resistance through selection for mobile resistance genes in animal intestines and can persist in lands fertilized with manure.[ADDIN EN.CITE ADDIN EN.CITE.DATA] There are concerns that manure with antimicrobials (and bioactive breakdown products) can select for or increase resistance in the soil, and alter the structure of the soil's microbial populations in different ways than antimicrobial-free manures.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Environments Exposed to Agricultural Wastes contaminated with AMR

Agricultural waste is an important fertilizer and it is usually processed prior to use. Manures are processed differently based on factors like the specific commodity, the size of the operation, the soil type, and the proximity to surface and ground water.[ADDIN EN.CITE <EndNote><Cite><Author>Durso</Author><Year>2016</Year><RecNum>173</RecNum><DisplayText><style face="superscript">[53]</style></DisplayText><record><rec-number>173</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523991470">173</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Durso, L. M.</author><author>Wedin, D. A.</author><author>Gilley, J. E.</author><author>Miller, D. N.</author><author>Marx, D. B.</author></authors></contributors><titles><title>Assessment of Selected Antibiotic Resistances in Ungrazed Native Nebraska Prairie Soils</title><secondary-title>J Environ Qual</secondary-title></titles><periodical><full-title>J Environ Qual</full-title></periodical><pages>454-62</pages><volume>45</volume><number>2</number><dates><year>2016</year><pub-dates><date>Mar</date></pub-dates></dates><isbn>0047-2425 (Print)
0047-2425 (Linking)</isbn><accession-num>27065391</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/27065391</url></related-urls></urls><electronic-resource-num>10.2134/jeq2015.06.0280</electronic-resource-num></record></Cite></EndNote>] In confined production systems, manures might be treated through aerobic (e.g., composting) or anaerobic digestion before they are used. These treatments can alter the distribution and abundance of antimicrobial-resistant bacteria and ARGs, but it is not known how effective they are at reducing environmental exposure.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Soils fertilized with animal manures or biosolids are enriched with antimicrobial-resistant microbes and ARGs when compared to soils that do not receive animal manures.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Once in the soil, antimicrobial-resistant microbes persist even in the absence of selective pressure from antimicrobials.[ADDIN EN.CITE <EndNote><Cite><Author>Kyselková</Author><Year>2015</Year><RecNum>184</RecNum><DisplayText><style face="superscript">[58]</style></DisplayText><record><rec-number>184</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523991986">184</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kyselková, Martina</author><author>Kotrbová, Lucie</author><author>Bhumibhamon, Gamonsiri</author><author>Chroňáková, Alica</author><author>Jirout, Jiří</author><author>Vrchotová, Naděžda</author><author>Schmitt, Heike</author><author>Elhottová, Dana</author></authors></contributors><titles><title>Tetracycline resistance genes persist in soil amended with cattle feces independently from chlortetracycline selection pressure</title><secondary-title>Soil Biology and Biochemistry</secondary-title></titles><periodical><full-title>Soil Biology and Biochemistry</full-title></periodical><pages>259-265</pages><volume>81</volume><keywords><keyword>Antibiotic resistance</keyword><keyword>Cattle feces</keyword><keyword>Chlortetracycline</keyword><keyword>Grassland soil</keyword><keyword>Tetracycline resistance genes</keyword><keyword>gene</keyword></keywords><dates><year>2015</year><pub-dates><date>2015/02/01/</date></pub-dates></dates><isbn>0038-0717</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0038071714004040</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.soilbio.2014.11.018</electronic-resource-num></record></Cite></EndNote>] Many studies show that manure amendments (additives that can harbor pathogens) may lead to altered resistant microbial communities in soils, [ADDIN EN.CITE ADDIN EN.CITE.DATA] with the potential to contaminate crops.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Commercial manure application rates that are calibrated to crop agronomic needs will include an estimate of 10^8 to 10^{13} copies of various ARGs per hectare, indicating a significant presence of resistant bacteria that would not be present otherwise.[ADDIN EN.CITE <EndNote><Cite><Author>Tien</Author><Year>2017</Year><RecNum>531</RecNum><DisplayText><s

tyle face="superscript">[64]</style></DisplayText><record><rec-number>531</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529499298">531</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Tien, Yuan-Ching</author><author>Li, Bing</author><author>Zhang, Tong</author><author>Scott, Andrew</author><author>Murray, Roger</author><author>Sabourin, Lyne</author><author>Marti, Romain</author><author>Topp, Edward</author></authors></contributors><titles><title>Impact of dairy manure pre-application treatment on manure composition, soil dynamics of antibiotic resistance genes, and abundance of antibiotic-resistance genes on vegetables at harvest</title><secondary-title>Science of The Total Environment</secondary-title></titles><periodical><full-title>Science of The Total Environment</full-title></periodical><pages>32-39</pages><volume>581-582</volume><keywords><keyword>Antibiotic resistance genes</keyword><keyword>Manure</keyword><keyword>Agricultural soil</keyword><keyword>Vegetables</keyword><keyword>Risk of exposure</keyword></keywords><dates><year>2017</year><pub-dates><date>2017/03/01/</date></pub-dates></dates><isbn>0048-9697</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0048969716328285</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.scitotenv.2016.12.138</electronic-resource-num></record></Cite></EndNote>]

Detecting carbapenem-resistant bacteria in feces or in the production environment of cattle, swine, and poultry is particularly concerning because widespread human exposure from the environment or food supply could potentially compromise this critically important class of antimicrobials.[ADDIN EN.CITE ADDIN EN.CITE.DATA] It is possible that livestock production or areas with manure applied can contaminate nearby surface and groundwater resources with resistant bacteria.[ADDIN EN.CITE ADDIN EN.CITE.DATA] The additional burden of ARGs needs to be assessed relative to the baseline level of resistance found in the environment.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Aquaculture

Aquaculture (the farming of fish and seafood) now supplies more than half of all seafood, equating to approximately 8% of global animal food proteins.[ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1111/faf.12152", "ISSN" : "14672979", "abstract" : "Fisheries and aquaculture production, imports, exports and equitability of distribu- tion determine the supply of aquatic food to people. Aquatic food security is achieved when a food supply is sufficient, safe,

sustainable, shockproof and sound: sufficient, to meet needs and preferences of people; safe, to provide nutritional benefit while pos- ing minimal health risks; sustainable, to provide food now and for future genera- tions; shock-proof, to provide resilience to shocks in production systems and supply chains; and sound, to meet legal and ethical standards for welfare of animals, people and environment. Here, we present an integrated assessment of these elements of the aquatic food system in the United Kingdom, a system linked to dynamic global net- works of producers, processors and markets. Our assessment addresses sufficiency of supply from aquaculture, fisheries and trade; safety of supply given biological, chemi- cal and radiation hazards; social, economic and environmental sustainability of pro- duction systems and supply chains; system resilience to social, economic and environmental shocks; welfare of fish, people and environment; and the authenticity of food. Conventionally, these aspects of the food system are not assessed collectively, so information supporting our assessment is widely dispersed. Our assessment reveals trade-offs and challenges in the food system that are easily overlooked in sectoral analyses of fisheries, aquaculture, health, medicine, human and fish welfare, safety and environment. We highlight potential benefits of an integrated, systematic and ongoing process to assess security of the aquatic food system and to predict impacts of social, economic and environmental change on food supply and demand.",
"author" : [{ "dropping-particle" : "", "family" : "Jennings", "given" : "Simon", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stentiford", "given" : "Grant D.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Leocadio", "given" : "Ana M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Jeffery", "given" : "Keith R.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Metcalfe", "given" : "Julian D.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katsiadaki", "given" : "Ioanna", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Auchterlonie", "given" : "Neil A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mangi", "given" : "Stephen C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pinnegar", "given" : "John K.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ellis", "given" : "Tim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Peeler", "given" : "Edmund J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Luisetti", "given" : "Tiziana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Baker-Austin", "given" : "Craig", "non-dropping-particle" : "",

"parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Brown", "given" : "Mary", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Catchpole", "given" : "Thomas L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Clyne", "given" : "Fiona J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dye", "given" : "Stephen R.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Edmonds", "given" : "Nathan J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hyder", "given" : "Kieran", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lee", "given" : "Janette", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lees", "given" : "David N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Morgan", "given" : "Owen C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "O'Brien", "given" : "Carl M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Oidtmann", "given" : "Birgit", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Posen", "given" : "Paulette E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Santos", "given" : "Ana Ribeiro", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Taylor", "given" : "Nick G H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Turner", "given" : "Andrew D.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Townhill", "given" : "Bryony L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Verner-Jeffreys", "given" : "David W.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Fish and Fisheries", "id" : "ITEM-1", "issued" : { "date-parts" : [["2016"]] }, "title" : "Aquatic food security: Insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment", "type" : "article-newspaper" }, "uris" : ["http://www.mendeley.com/documents/?uuid=6a4bde17-8466-4d8c-9280-283dd0df3c42"] }, { "id" : "ITEM-2", "itemData" : { "ISBN" : "978-92-5-106675-1", "ISSN" : "1020-5489", "author" : [{ "dropping-particle" : "", "family" : "FAO", "given" : "", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "id" : "ITEM-2", "issued" : { "date-parts" : [["2016"]] }, "number-of-pages" : "200", "publisher-place" : "Rome", "title" : "The State of World Fisheries and Aquaculture. Contributing to food security and nutrition for all.", "type" : "book" }, "uris" : [

"http://www.mendeley.com/documents/?uuid=5246ffbf-029d-49b2-bc61-a8f2609b699d"] }], "mendeley" : { "formattedCitation" : "(Jennings et al. 2016; FAO 2016)", "plainTextFormattedCitation" : "(Jennings et al. 2016; FAO 2016)", "previouslyFormattedCitation" : "(Jennings et al. 2016; FAO 2016)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }] In 2015, total aquaculture production worldwide was 76.6 million tonnes (excluding aquatic plants and non-food products). The top ten aquaculture producers included:[ADDIN EN.CITE <EndNote><Cite><Author>FAO</Author><Year>2017</Year><RecNum>517</RecNum><DisplayText><style face="superscript">[74]</style></DisplayText><record><rec-number>517</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527787464">517</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>FAO</author></authors></contributors><titles><title> STATISTICS FISHERIES AND AQUACULTURE STATISTICS STATISTIQUES DES PÊCHES</title></titles><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>]

- China (47.6 million tonnes)
- India (5.2 million tonnes)
- Indonesia (4.3 million tonnes)
- Vietnam (3.4 million tonnes)
- Bangladesh (2.1 million tonnes)
- Norway (1.4 million tonnes)
- Egypt (1.2 million tonnes)
- Chile (1 million tonnes)
- Myanmar (1 million tonnes)
- Thailand (0.9 million tonnes)

Antimicrobials are used worldwide in aquaculture, particularly in intensive rearing systems, to control disease[ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "author" : [{ "dropping-particle" : "", "family" : "Smith", "given" : "P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "id" : "ITEM-1", "issue" : "1", "issued" : { "date-parts" : [["2008"]] }, "page" : "243-264", "title" : "Antimicrobial resistance in aquaculture The use of antimicrobials in aquaculture", "type" : "article-journal", "volume" : "27" }, "uris" : ["http://www.mendeley.com/documents/?uuid=aa333a81-7bde-455f-9bf7-110319e22eaa"] }], "mendeley" : { "formattedCitation" : "(Smith 2008)", "plainTextFormattedCitation" : "(Smith 2008)", "previouslyFormattedCitation" : "(Smith 2008)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }]. These are generally administered in feed or occasionally through bath treatments. Overall, estimates of antimicrobial use in aquaculture are difficult to determine, as sales and use records are often incomplete or missing. The most complete antimicrobial use information is for high value aquatic species farmed in high-income countries, but this information does not represent overall estimates and patterns of use.[ADDIN EN.CITE <EndNote><Cite><Author>Henriksson</Author><Year>2017</Year><RecNum>501</RecNum><DisplayText><style face="superscript">[75]</style></DisplayText><record><rec-number>501</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527784726">501</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Henriksson, Patrik J. G.</author><author>Rico, Andreu</author><author>Troell, Max</author><author>Klinger, Dane H.</author><author>Buschmann, Alejandro H.</author><author>Saksida, Sonja</author><author>Chadag, Mohan V.</author><author>Zhang, Wenbo</author></authors></contributors><titles><title>Unpacking factors influencing antimicrobial use in global aquaculture and their implication for management: a review from a systems perspective</title><secondary-title>Sustainability Science</secondary-title></titles><periodical><full-title>Sustainability Science</full-title></periodical><dates><year>2017</year><pub-dates><date>November 18</date></pub-dates></dates><isbn>1862-4057</isbn><label>Henriksson2017</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1007/s11625-017-0511-8</url></related-urls></urls><electronic-resource-num>10.1007/s11625-017-0511-8</electronic-resource-num></record></Cite></EndNote>] In these high-income countries, antimicrobial use is often tightly regulated under similar systems as those used for terrestrial animals. However, even in countries where antimicrobial use is regulated, there can be considerable variation in use. For example, Smith et al.[ADDIN EN.CITE <EndNote><Cite><Author>Smith</Author><Year>2008</Year><RecNum>512</RecNum><DisplayText><style face="superscript">[76]</style></DisplayText><record><rec-number>512</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527787241">512</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Smith, P.</author></authors></contributors><auth-address>Department of Microbiology, National University of Ireland Galway, Galway, Ireland. peterrsmith@eircom.net</auth-address><titles><title>Antimicrobial resistance in aquaculture</title><secondary-title>Rev Sci Tech</secondary-title></titles><periodical><full-title>Rev Sci Tech</full-title><abbr-1>Revue scientifique et technique (International Office of Epizootics)</abbr-

1></periodical><pages>243-64</pages><volume>27</volume><number>1</number><keywords><keyword>Animals</keyword><keyword>Anti-Bacterial Agents/adverse effects/*therapeutic use</keyword><keyword>*Aquaculture</keyword><keyword>Dose-Response Relationship, Drug</keyword><keyword>*Drug Resistance, Bacterial</keyword><keyword>Fish Diseases/*drug therapy/*microbiology</keyword><keyword>Fishes</keyword><keyword>Microbial Sensitivity Tests/veterinary</keyword><keyword>*Public Health</keyword><keyword>Risk Assessment</keyword><keyword>Treatment Outcome</keyword></keywords><dates><year>2008</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>0253-1933 (Print)
0253-1933 (Linking)</isbn><accession-num>18666490</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/18666490</url></related-urls></urls></record></Cite></EndNote>] estimated that only 1mg of antimicrobial agents was used per kg of production in Norway (predominately for their greater than 1 million tonnes of Atlantic salmon production). Chile (the second largest producer of Atlantic salmon) used more than 560 tonnes of antimicrobials in 2015, which equates to more than 600 mg per kg of salmon production[ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "abstract" : "Aquaculture in Chile has been practiced since the 1920s; however, it was not until the 1990s that aquaculture became an important sector here. Important species in Chilean aquaculture include salmonids, algae, mollusks, and turbot. Salmonids are the dominant species in Chilean aquaculture for both harvest volume and export value, their production reaching greater than 800-thousand tons in 2015. However, this growth has been accompanied by an increase in disease presence, requiring greater drug use to control. This increase in drug use is an environmental and public health concern for the authorities, the salmon industry itself, and the destination markets. In this chapter, we review the literature on drug use, antibiotic resistance, regulatory framework, and alternatives, with focus on Chile.", "author" : [{ "dropping-particle" : "", "family" : "Lozana", "given" : "I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Diaz", "given" : "N F", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Munoz", "given" : "S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "C", "given" : "Riquelme", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "chapter-number" : "3", "container-title" : "Antibiotic Use in Animals", "editor" : [{ "dropping-particle" : "", "family" : "Savic", "given" : "Sara", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "id" : "ITEM-1", "issued" : { "date-parts" : [["2018"]] }, "page" : "25-44", "publisher" : "IntechOpen Limited", "publisher-place" : "London, UK", "title" : "Antibiotics in Chilean aquaculture: a review", "type" : "chapter" }, "uris" : ["http://www.mendeley.com/documents/?uuid=b265da37-21e3-4187-b8d0-4e4effbaaad1"] }], "mendeley" : { "formattedCitation" : "(Lozana et al. 2018)", "plainTextFormattedCitation" : "(Lozana et al. 2018)", "previouslyFormattedCitation" : "(Lozana et al. 2018)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }]. This high antimicrobial use in Chile is associated with control of outbreaks of piscirickettsiosis caused by the bacterium *Piscirickettsia salmonis*.

The number of different antimicrobials authorized for use in high- and middle-income countries is typically very limited. For instance, in the U.K. there are only three antimicrobial products with Marketing Authorizations for use in farmed salmonids: florfenicol, oxytetracycline, and amoxicillin.

For other major producers, like many countries in South East Asia, antimicrobial use estimates are difficult to compile because there are no (or very limited) efforts to collect antimicrobial use or other relevant data, such as sales. Data is particularly difficult to gather since production is often broken up among many small-scale subsistence-level enterprises. The limited available data from countries in Asia are often based on extrapolations from isolated farmer surveys of antimicrobial use, but total antimicrobial use is likely to be considerable. For instance, based on analysis of surface water samples for antimicrobial residues, it was estimated that approximately 5,800 tonnes of enrofloxacin, 1,800 tonnes of sulphadiazine, 12,300 tonnes of sulphamethoxazole, and 6,400 tonnes of trimethoprim are discharged into the Mekong Delta every year[ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1371/journal.pone.0131855", "ISSN" : "1932-6203", "PMID" : "26135396", "abstract" : "The Mekong Delta in Vietnam has seen a rapid development and intensification of aquaculture in the last decades, with a corresponding widespread use of antibiotics. This study provides information on current antibiotic use in freshwater aquaculture, as well as on resulting antibiotic concentrations in the aquatic environment of the Mekong Delta. Two major production steps, fish hatcheries and mature fish cultivation, were surveyed (50 fish farm interviews) for antibiotic use. Different water sources, including surface water, groundwater and piped water (164 water samples) were systematically screened for antibiotic residues. To better understand antibiotic fate under tropical conditions, the dissipation behavior of selected antibiotics in the aquatic environment was investigated for the first time in mesocosm experiments. None of the investigated antibiotics were detected in groundwater and piped water samples. Surface water, which is still often used for drinking and domestic

purposes by local populations, contained median concentrations of 21 ng L-1 sulfamethoxazole (SMX), 4 ng L-1 sulfadiazine (SDZ), 17 ng L-1 trimethoprim (TRIM), and 12 ng L-1 enrofloxacin (ENRO). These concentrations were lower than the predicted no effect concentrations (PNECs) and minimum inhibitory concentrations (MICs), suggesting limited antibiotic-related risk to aquatic ecosystems in the monitored systems. The dissipation half-lives of the studied antibiotics ranged from <1 to 44 days, depending on the availability of sunlight and sediment. Among the studied antibiotics TRIM was the most persistent in water systems. TRIM was not susceptible to photodegradation, while the dissipation of ENRO and SDZ was influenced by photolysis. The recorded dissipation models gave good predictions of the occurrence and concentrations of TRIM, ENRO and SDZ in surface water. In summary, the currently measured concentrations of the investigated antibiotics are unlikely to cause immediate risks to the aquatic environment, yet the persistence of these antibiotics is of concern and might lead to chronic exposure of aquatic organisms as well as humans.", "author" : [{ "dropping-particle" : "", "family" : "Nguyen Dang Giang", "given" : "Chau", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sebesvari", "given" : "Zita", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Renaud", "given" : "Fabrice", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rosendahl", "given" : "Ingrid", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hoang Minh", "given" : "Quang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Amelung", "given" : "Wulf", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "PloS one", "id" : "ITEM-1", "issue" : "7", "issued" : { "date-parts" : [["2015", "1", "2"]] }, "page" : "e0131855", "publisher" : "Public Library of Science", "title" : "Occurrence and Dissipation of the Antibiotics Sulfamethoxazole, Sulfadiazine, Trimethoprim, and Enrofloxacin in the Mekong Delta, Vietnam.", "type" : "article-journal", "volume" : "10" }, "uris" : ["http://www.mendeley.com/documents/?uuid=12b0eafd-5e0c-4490-b0fa-13ff96c330c6"] }], "mendeley" : { "formattedCitation" : "(Nguyen Dang Giang et al. 2015)", "plainTextFormattedCitation" : "(Nguyen Dang Giang et al. 2015)", "previouslyFormattedCitation" : "(Nguyen Dang Giang et al. 2015)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }].[ADDIN EN.CITE <EndNote><Cite><Author>Nguyen Dang Giang</Author><Year>2015</Year><RecNum>507</RecNum><DisplayText><style face="superscript">[77]</style></DisplayText><record><rec-number>507</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527785837">507</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>Nguyen Dang Giang, Chau</author><author>Sebesvari,
Zita</author><author>Renaud, Fabrice</author><author>Rosendahl, Ingrid</author><author>Hoang
Minh, Quang</author><author>Amelung,
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resource-num>10.1371/journal.pone.0131855</electronic-resource-
num></record></Cite></EndNote>] Although this includes discharge from terrestrial livestock production, major sources were also from large shrimp and fish culture systems based in this region. Survey results also revealed that catfish farmers in this region were using up to 17 different antimicrobial agent treatments, with an estimated 93mg of antimicrobial agents used per kg harvested fish. The antimicrobial agents that had the highest contribution to this amount were sulfamethoxazole, cephalexin, amoxicillin, florfenicol, and enrofloxacin.[ADDIN EN.CITE <EndNote><Cite><Author>Nguyen
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There is debate as to whether the overall use of antimicrobial agents in aquaculture represents a significant fraction of use in all food animals. Regardless, there is concern that use, if not practiced sustainably, could contaminate the environment and drive resistance development in key pathogens that affect fish and shellfish. This could cause a decrease in productivity and negatively affect the welfare of producers. The aquatic environment, where these animals are reared, likely has a role in the development and dissemination of AMR. It is possible that aquaculture operations contribute to this process.

Antimicrobial-resistant microbes usually found in humans can be discharged into the aquatic environment from sources like washoff from agricultural holdings and from treated and untreated human sewage. Aquaculture rearing facilities might also act as reservoirs for these organisms and the mobile resistance genetic elements they carry. The discharged microbes could potentially transfer into the aquatic microbial communities pathogenic and non-pathogenic microbes associated with farmed aquatic animals. There are some studies demonstrating that fish and shellfish pathogens have acquired resistance genes and associated mobile elements that are similar to resistance from clinical bacterial isolates. This demonstrates that there were likely common origins (pathogens transferred from humans to fish)[ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1128/AAC.01312-08", "ISBN" : "1098-6596 (Electronic)\\r0066-4804 (Linking)", "ISSN" : "00664804", "PMID" : "19029319", "abstract" : "Florfenicol (FFC) has recently been approved by the Food and Drug Administration for the treatment of several bacterial diseases of cultured fish species in the United States, including enteric septicemia of catfish (ESC) caused by Edwardsiella ictaluri. The FFC-resistant E. ictaluri strain (M07-1) described herein was isolated from a moribund catfish obtained from the Thad Cochran National Warmwa- ter Aquaculture Research Center (Stoneville, MS) inMay of 2007 and was confirmed to be E. ictaluri by 16S rRNA gene sequencing (6). Fish showing signs of ESC were examined for FFC-resistant E. ictaluri because losses due to ESC per- sisted in this population despite FFC treatment", "author" : [{ "dropping-particle" : "", "family" : "Welch", "given" : "Timothy J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Evenhuis", "given" : "Jason", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "White", "given" : "David G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "McDermott", "given" : "Patrick F.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Harbottle", "given" : "Heather", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Miller", "given" : "Ron A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Griffin", "given" : "Matt", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wise", "given" : "David", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Antimicrobial Agents and Chemotherapy", "id" : "ITEM-1", "issued" : { "date-parts" : [["2009"]] }, "page" : "845-846", "title" : "IncA/C plasmid-mediated florfenicol resistance in the catfish pathogen Edwardsiella ictaluri", "type" : "article", "volume" : "53" }, "uris" : ["http://www.mendeley.com/documents/?uuid=7a69b8a8-98ab-4a2f-9a71-b838578119b8"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1128/AAC.01312-08", "ISSN" : "1098-6596", "PMID" : "19029319", "author" : [{ "dropping-particle" : "", "family" : "Welch", "given" : "Timothy J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Evenhuis", "given" : "Jason", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "White", "given" : "David G", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "McDermott", "given" : "Patrick F", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Harbottle", "given" : "Heather", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" :

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Antibiotic and mercury resistance genes were carried by a conjugative IncA/C plasmid. Three distinct antibiotic resistance cassettes were characterized; first a class I integron containing an aadA7 gene encoding for an aminoglycoside-3'-adenyltransferase, the second cassette showed 99.9% nucleotide sequence homology to a cassette previously identified in the Salmonella enterica IncA/C plasmid pSN254, containing floR, tetA, sulII and strA/strB sequences. The third cassette showed 100% nucleotide sequence similarity to a transposon-like element, containing a bla(CMY-2) beta-lactamase in association with sugE and blc sequences. This element is known to be widely distributed among clinical and food-borne Salmonella and other Enterobacteriaceae throughout Asia and the United States. Mercury resistance was linked to the presence of a mer operon that showed 100% nucleotide sequence homology to the mer operon carried by plasmid pSN254.\n\nCONCLUSIONS: Each MDR A. salmonicida isolate carried the same plasmid, which was related to plasmid pSN254. This is the first report of plasmid-mediated florfenicol-resistant A. salmonicida in North America. In addition, it is the first report of a plasmid-associated AmpC beta-lactamase sequence in a member of the Aeromonadaceae.", "author" : [{ "dropping-particle" : "", "family" : "McIntosh", "given" : "Douglas", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cunningham", "given" : "Michelle", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ji", "given" : "Baijing", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fekete", "given" : "Frank a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Parry", "given" : "Erin M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Clark", "given" : "Sarah E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zalinger", "given" : "Zachary B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gilg", "given" : "Ilana C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Danner", "given" : "G Russell", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Johnson", "given" : "Keith a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Beattie", "given" : "Mike", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ritchie", "given" : "Rachael", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "The Journal of antimicrobial chemotherapy", "id" : "ITEM-3", "issue" : "6", "issued" : { "date-parts" : [["2008", "6"]] }, "page" : "1221-8", "title" : "Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of Aeromonas salmonicida subsp. salmonicida is associated with carriage of an IncA/C plasmid similar to the Salmonella enterica plasmid pSN254.", "type" : "article-journal", "volume" : "61" }, "uris" : ["http://www.mendeley.com/documents/?uuid=372a0339-d3e1-47f7-9e75-0fefdec41721"] }], "mendeley" : { "formattedCitation" : "(Timothy J. Welch et al. 2009; Timothy J Welch et al. 2009; McIntosh et al. 2008)", "plainTextFormattedCitation" : "(Timothy J. Welch et al. 2009; Timothy J Welch et al. 2009; McIntosh et al. 2008)", "previouslyFormattedCitation" : "(Timothy J. Welch et al. 2009; Timothy J Welch et al. 2009; McIntosh et al. 2008)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }].[ADDIN EN.CITE ADDIN EN.CITE.DATA] Transfer in the other direction (fish pathogens to humans) is also theoretically possible and there are potential exposure routes (e.g., handling and consumption). Conjugation (a form of sexual reproduction for unicellular organisms) of resistance plasmids was successfully performed in the laboratory in raw salmon between a resistant strain of the fish pathogenic bacterium *Aeromonas salmonicida* subspecies *salmonicida* and a susceptible *E. coli* strain of human origin. However, there is no evidence of a human disease-causing agent acquiring resistance from aquaculture origins. Cooking food before eating helps to minimize this risk. More information is needed to understand the risk of consuming raw seafood (e.g., oysters or sushi). Additionally, bivalves are also worthy of study because they are filter feeders, meaning they tend to bioconcentrate (store) bacteria in the water column, including resistant human pathogens.

Antimicrobials can also be used in large quantities to support rearing ornamental fish (pets) and other aquatic species not meant for eating.[ADDIN EN.CITE ADDIN EN.CITE.DATA] It has been shown that the amount of resistance in traded ornamental fish species can be very high. Resistant pathogens and ARGs could transfer from fish to people since owners keep the fish species nearby and handle them. There have been some limited reports linking human bacterial infections with exposure to ornamental fish.[ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "author" : [{ "dropping-particle" : "", "family" : "Musto", "given" : "Jennie", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kirk", "given" : "Martyn", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lightfoot", "given" : "Diane", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Combs", "given" : "Barry G", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mwanri", "given" : "Lillian", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Communicable Diseases Intelligence", "id" : "ITEM-1", "issue" : "2", "issued" : { "date-parts" : [["2006"]] }, "page" : "222-227", "title" : "Multi-drug resistant Salmonella Java infections acquired from tropical fi sh", "type" : "article-journal", "volume" : "30" }, "uris" : ["http://www.mendeley.com/documents/?uuid=d4a3d027-3712-4f9d-8313-d4d82e0020a6"] }], "mendeley" : { "formattedCitation" : "(Musto et al. 2006)", "plainTextFormattedCitation" : "(Musto et al. 2006)", "previouslyFormattedCitation" : "(Musto et al. 2006)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }]

However, the actual risks to human and animal health are not well described or understood. More information is needed on antimicrobial use in aquaculture generally, including the quantities and types used, and the reasons antimicrobials are applied instead of applying other control methods. More information is also needed about the levels and rates of resistance change in microbes (pathogens and commensals) associated with aquaculture production systems, especially in the tropical and subtropical production areas, and the risks posed to consumers and farmed fish. This will require developing strategies to effectively assess the problem at a national and international level. The World Organisation for Animal Health (OIE) aquatic animal code provides recommendations, available online at [HYPERLINK "http://www.oie.int/index.php?id=171&L=0&htmfile=titre_1.6.htm"].

Alternatives to Antimicrobial use in Aquafarming

Efforts have been made to encourage the use of alternative control methods instead of using antimicrobials. For example, Norway, Scotland, and all the other major production areas (except Chile) have successfully implemented vaccination-based control strategies for the rainbow trout sector and the Atlantic salmon industry. Vaccinations are also widely used in sea bream and seabass industries in Southern Europe. Vaccines have been less successful in other, often less profitable, finfish aquaculture sectors presumably because development and administration costs remain high. Also, although vaccines can efficiently prevent bacterial disease outbreaks in finfish, they are not as effective for crustaceans or mollusks since these animals do not have an adaptive immune system.

Another major method of reducing antimicrobial use includes improving biosecurity and the quality of the rearing environment. There are less diseases when there is good water quality and balanced stocking densities because the fish are less stressed.[ADDIN EN.CITE <EndNote><Cite><Author>H.</Author><Year>2017</Year><RecNum>605</RecNum><DisplayText><style face="superscript">[82]</style></DisplayText><record><rec-number>605</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1531229636">605</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Stevens C. H.</author><author>Croft D. P.</author><author>Paull G. C.</author><author>Tyler C. R.</author></authors></contributors><titles><title>Stress and welfare in ornamental fishes: what can be learned from aquaculture?</title><secondary-title>Journal of Fish Biology</secondary-title></titles><periodical><full-title>Journal of Fish Biology</full-title></periodical><pages>409-428</pages><volume>91</volume><number>2</number><dates><year>2017</year></dates><urls><related-urls><url>https://onlinelibrary.wiley.com/doi/abs/10.1111/jfb.13377</url></related-urls></urls><electronic-resource-num>doi:10.1111/jfb.13377</electronic-resource-num></record></Cite></EndNote>] Where practical, implementing fallowing (gaps in production) between rearing different fish cohorts can also reduce disease burdens in farms. These systems can be implemented at various levels, from the local farm level to the national level, through area management plans and other structures.

Better disease diagnostics and early warning systems for the emergence of disease can also help reduce the need for antimicrobials. It is recognized that diagnosis and treatment is often initiated too late when high levels of antimicrobials are already in use. Additionally, many diseases cause a lack of appetite, further reducing the effectiveness of feed-administered antimicrobial treatments.

When alternatives are not available or effective, targeted and appropriate regulation to control the sales and administration of antimicrobials, backed up by product certification schemes, can help reduce the

use of antimicrobials.[ADDIN EN.CITE
<EndNote><Cite><Author>Henriksson</Author><Year>2017</Year><RecNum>501</RecNum><DisplayText><style face="superscript">[75]</style></DisplayText><record><rec-number>501</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527784726">501</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Henriksson, Patrik J. G.</author><author>Rico, Andreu</author><author>Troell, Max</author><author>Klinger, Dane H.</author><author>Buschmann, Alejandro H.</author><author>Saksida, Sonja</author><author>Chadag, Mohan V.</author><author>Zhang, Wenbo</author></authors></contributors><titles><title>Unpacking factors influencing antimicrobial use in global aquaculture and their implication for management: a review from a systems perspective</title><secondary-title>Sustainability Science</secondary-title></titles><periodical><full-title>Sustainability Science</full-title></periodical><dates><year>2017</year><pub-dates><date>November 18</date></pub-dates></dates><isbn>1862-4057</isbn><label>Henriksson2017</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1007/s11625-017-0511-8</url></related-urls></urls><electronic-resource-num>10.1007/s11625-017-0511-8</electronic-resource-num></record></Cite></EndNote>]

B. How should the presence of AMR in the environment be measured? Do methods differ if testing for attribution (e.g., tracking resistant pathogens to a source like hospital, septic systems, or farms)? Can these methods be standardized and used to monitor the impact of mitigation measure?

Methods for Detecting and Enumerating Antimicrobial-resistant Pathogens and ARGs

Many methods are available to detect antimicrobial-resistant pathogens and ARGs in environmental samples (e.g., soil, water, or manure) (Table 1). There is no single best method to detect AMR or ARGs, and the methods vary in sensitivity, cost, and technical requirements. The method that is best for a particular place, time, and question should be used. The following includes the advantages and limitations of each method.

Culture-based Methods

Microbial culture, where microorganisms are grown and counted in the laboratory, has historically been the gold-standard approach to detect antimicrobial-resistant pathogens. Culture-based methods are inexpensive, quantitative, and easily transferred from clinical settings. Culture-based detection of AMR in environmental samples uses a variety of selective or screening media to isolate the bacteria of interest. Commercially available media exist that target a wide variety of bacteria. Equipment requirements are minimal, making this approach well suited to low resource settings. In contrast to molecular methods, culture-based detection ensures that the bacteria detected are viable and meet regulatory cutoffs for resistance. Antimicrobial-resistant bacteria can be isolated directly from samples by including antimicrobials in the selective media, and if parallel tests are conducted without antimicrobials then this will allow estimation of the proportion of a bacterial community that is resistant.

Culture-based approaches also have substantial limitations for environmental microbiology. Most bacteria from the natural environment cannot be cultured in the lab, a limitation that is particularly profound in environmental samples. In addition, many bacteria can enter a state where the microbe is alive but does not multiply under environmental stress. For bacteria that can be cultured, the process can be time-consuming, requiring long incubations, multiple steps, and confirmatory analyses. Methods used to store the samples and the duration of storage can both strongly influence recovery and quantification of the target organisms. Perhaps the greatest limitation of culture-based methods is that they are not high throughput. Given the bacterial diversity of environmental samples, decisions must be made about what types of bacteria need to be recovered from culture and what types of resistance need to be detected. These decisions help to refine laboratory test schemes.

Broth microdilution, in which an isolate is exposed to increasing antimicrobial concentrations to identify the level of that antimicrobial that inhibits growth, is the preferred method to determine whether an isolate is susceptible or resistant to a level of a drug, defined by the minimum inhibitory concentration (MIC) towards that drug. Standardized protocols, as well as cutoffs for assessing resistance or susceptibility, are available. MIC determination also allows monitoring of stepwise increases in resistance ("MIC creep") that may be missed with methods that return only susceptible or resistant determinations. However, MIC cutoffs to determine susceptibility are based on clinical treatment outcomes and may not be appropriate for environmental monitoring. They also perform at clinically relevant standard temperatures, which may not reflect environmental conditions. Suggestions include using epidemiological cutoffs based on population MIC distributions or ecological cutoffs based on arithmetic MIC distributions. Disk diffusion is a simpler method of measuring antimicrobial susceptibility that can be used to determine resistance and estimate MICs. Interpretation of disk diffusion results into susceptible and resistant categories suffers from the same limitations as MIC testing.

Molecular Methods

Molecular methods are used to genetically characterize microbial isolates (pathogens and commensals). They are used to detect and track ARGs, and enumerate microbes (determine the number of individual viable microbes in a sample) from environmental samples. Targets include the ARGs, determinants for genus and species identification, as well as genes like integrases, insertion sequences, or plasmid-associated genes that are often associated with horizontal gene transfer. If well designed, molecular methods are robust, economical, and easy to use,[ADDIN EN.CITE <EndNote><Cite><Author>Storteboom</Author><Year>2010</Year><RecNum>530</RecNum><DisplayText><style face="superscript">[83]</style></DisplayText><record><rec-number>530</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529499197">530</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Storteboom, H.</author><author>Arabi, M.</author><author>Davis, J. G.</author><author>Crimi, B.</author><author>Pruden, A.</author></authors></contributors><titles><title>Identification of Antibiotic-Resistance-Gene Molecular Signatures Suitable as Tracers of Pristine River, Urban, and Agricultural Sources</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>1947-1953</pages><volume>44</volume><number>6</number><dates><year>2010</year><pub-dates><date>2010/03/15</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/es902893f</url></related-urls></urls><electronic-resource-num>10.1021/es902893f</electronic-resource-num></record></Cite></EndNote>] but several factors have limited the widespread use of molecular methods for measuring resistance in environmental samples to date, including expense, complexity of assay development, and accessibility of required instruments. However, these technologies are decreasing in price and becoming more widespread in microbiological laboratories.

The polymerase chain reaction (PCR) is a technique used to make copies of a target piece of DNA, and is the foundation for many molecular methods. Standard PCR methods are able to provide presence/absence information for a target gene, but do not provide information on what proportion of a sample is resistant.

Quantitative PCR (qPCR) assays allow for enumeration of the target gene, but the limit of detection can pose a challenge particularly when analyzing environmental samples that may contain PCR inhibitors (i.e., complex organic acids and metals often found environmental samples, but rarely found in clinical samples) and low quantities of the target gene. Furthermore, qPCR methods are more expensive than standard PCR, and may rely on comparison with a standard to enumerate. This makes it difficult to compare quantitative data between laboratories. However, having greater quantitative data with rapid turnaround times to evaluate the impact of interventions on AMR makes qPCR a common choice for studies evaluating AMR in field studies.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Commercial companies use the qPCR platform for products designed to quantify multiple ARG targets simultaneously in 96- or 384-well formats.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Assays for multiple targets can be less sensitive than assays for a single target because reactions are not optimized for each individual target. Alternatively, Droplet Digital™ PCR uses new technology to aerosolize a sample into thousands of individual droplets, which are individually assayed for ARGs using standard qPCR methods.[ADDIN EN.CITE
<EndNote><Cite><Author>Cavé</Author><Year>2016</Year><RecNum>534</RecNum><DisplayText><style face="superscript">[87]</style></DisplayText><record><rec-number>534</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529499721">534</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Cavé, Laura</author><author>Brothier, Elisabeth</author><author>Abrouk, Danis</author><author>Bouda, Panignimyandé Salomon</author><author>Hien, Edmond</author><author>Nazaret, Sylvie</author></authors></contributors><titles><title>Efficiency and sensitivity of the digital droplet PCR for the quantification of antibiotic resistance genes in soils and organic residues</title><secondary-title>Applied Microbiology and Biotechnology</secondary-title></titles><periodical><full-title>Applied Microbiology and Biotechnology</full-title></periodical><pages>10597-10608</pages><volume>100</volume><number>24</number><dates><year>2016</year><pub-dates><date>December 01</date></pub-dates></dates><isbn>1432-0614</isbn><label>Cavé2016</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1007/s00253-016-7950-5</url></related-urls></urls><electronic-resource-

num>10.1007/s00253-016-7950-5</electronic-resource-num></record></Cite></EndNote>] It eliminates the limit of quantification issue, and is more accurate than qPCR. Droplet Digital™ PCR does not have the same barriers as PCR and qPCR, but the technology is new to environmental microbiology and method development is still in its infancy.[ADDIN EN.CITE <EndNote><Cite><Author>Rački</Author><Year>2014</Year><RecNum>598</RecNum><DisplayText><style face="superscript">[88]</style></DisplayText><record><rec-number>598</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1530547795">598</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Rački, Nejc</author><author>Dreo, Tanja</author><author>Gutierrez-Aguirre, Ion</author><author>Blejec, Andrej</author><author>Ravnikar, Maja</author></authors></contributors><titles><title>Reverse transcriptase droplet digital PCR shows high resilience to PCR inhibitors from plant, soil and water samples</title><secondary-title>Plant Methods</secondary-title></titles><periodical><full-title>Plant Methods</full-title></periodical><pages>42</pages><volume>10</volume><number>1</number><dates><year>2014</year><pub-dates><date>December 31</date></pub-dates></dates><isbn>1746-4811</isbn><label>Rački2014</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1186/s13007-014-0042-6</url></related-urls></urls><electronic-resource-num>10.1186/s13007-014-0042-6</electronic-resource-num></record></Cite></EndNote>]

A second set of molecular methods relies on DNA sequencing, which provides detailed genetic information. In amplicon sequencing (a targeted sequencing approach), a single gene (often the 16S rRNA gene) is amplified using PCR, and the resulting amplicons are sequenced. This captures the many varieties of the gene in the sample. DNA sequencing can also target functional genes, like ARGs. A second sequencing approach that incorporates an initial PCR step is epicPCR, which allows for sequencing whole communities in a way that links the 16S and ARGs for each cell, allowing attribution of the resistance to a specific bacterium. The method was designed to address questions in microbial ecology, and has been demonstrated to work in environmental samples.[ADDIN EN.CITE <EndNote><Cite><Author>Spencer</Author><Year>2016</Year><RecNum>535</RecNum><DisplayText><style face="superscript">[89]</style></DisplayText><record><rec-number>535</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529499876">535</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Spencer, Sarah J.</author><author>Tamminen, Manu

V.</author><author>Preheim, Sarah P.</author><author>Guo, Mira T.</author><author>Briggs, Adrian W.</author><author>Brito, Ilana L.</author><author>A Weitz, David</author><author>Pitkänen, Leena K.</author><author>Vigneault, Francois</author><author>Virta, Marko PJuhani</author><author>Alm, Eric J.</author></authors></contributors><titles><title>Massively parallel sequencing of single cells by epicPCR links functional genes with phylogenetic markers</title><secondary-title>The ISME Journal</secondary-title></titles><periodical><full-title>The Isme Journal</full-title></periodical><pages>427-436</pages><volume>10</volume><number>2</number><dates><year>2016</year><pub-dates><date>09/22
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Molecular approaches to AMR determination in bacterial isolates include whole genome sequencing (WGS) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS). WGS can be used to detect known ARGs in isolates and the predicted resistance has been shown to correlate well with phenotypic resistance in clinical isolates.[ADDIN EN.CITE ADDIN EN.CITE.DATA] WGS is now commonly used for public health AMR surveillance efforts, but its accuracy has not been evaluated for environmental bacteria. Currently, WGS is only able to determine whether resistance genes are present, not the level of resistance. Methods to estimate MICs from WGS data are being developed.[ADDIN EN.CITE <EndNote><Cite><Author>Nguyen</Author><Year>2018</Year><RecNum>540</RecNum><DisplayText><style face="superscript">[93]</style></DisplayText><record><rec-number>540</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529500214">540</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Nguyen, Marcus</author><author>Brettin, Thomas</author><author>Long, S. Wesley</author><author>Musser, James M.</author><author>Olsen, Randall J.</author><author>Olson, Robert</author><author>Shukla, Maulik</author><author>Stevens, Rick L.</author><author>Xia, Fangfang</author><author>Yoo, Hyunseung</author><author>Davis, James J.</author></authors></contributors><titles><title>Developing an in silico minimum inhibitory concentration panel test for Klebsiella pneumoniae</title><secondary-title>Scientific Reports</secondary-title></titles><periodical><full-title>Scientific Reports</full-title></periodical><pages>421</pages><volume>8</volume><number>1</number><dates><year>2018</year><pub-dates><date>2018/01/11</date></pub-dates></dates><isbn>2045-2322</isbn><urls><related-urls><url>https://doi.org/10.1038/s41598-017-18972-w</url></related-urls></urls><electronic-resource-num>10.1038/s41598-017-18972-w</electronic-resource-num></record></Cite></EndNote>] Moreover, WGS can only detect known resistance genes or those with similarity to known resistance genes. WGS provide inferences on genetic mobility of ARGs or ARGs that are genetically interlinked, which can be critical for estimating the human health risk of exposure and the risk of horizontal transmission.

MALDI-ToF MS is a quick and reliable approach for bacterial identification, even for hard to culture organisms.[ADDIN EN.CITE <EndNote><Cite><Author>Biswas</Author><Year>2013</Year><RecNum>275</RecNum><DisplayText><style face="superscript">[94]</style></DisplayText><record><rec-number>275</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524057683">275</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Biswas, Silpak</author><author>Rolain, Jean-Marc</author></authors></contributors><titles><title>Use of MALDI-TOF mass spectrometry for identification of bacteria that are difficult to culture</title><secondary-title>Journal of Microbiological Methods</secondary-title></titles><periodical><full-title>Journal of Microbiological Methods</full-title></periodical><pages>14-24</pages><volume>92</volume><number>1</number><keywords><keyword>Anaerobes</keyword><keyword>Mass spectrometry</keyword><keyword>Fastidious bacteria</keyword><keyword>Slow growing bacteria</keyword><keyword>Proteomic profiling</keyword><keyword>16S rRNA gene sequencing</keyword></keywords><dates><year>2013</year><pub-dates><date>2013/01/01/</date></pub-dates></dates><isbn>0167-7012</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0167701212003478</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.mimet.2012.10.014</electronic-resource-num></record></Cite></EndNote>] Test modifications have been developed to improve sensitivity and accuracy of MALDI-ToF MS to, for example, detect antimicrobial-resistant phenotypes by detection of antimicrobial-resistant proteins, modification or breakdown of the target antimicrobial, or inhibition of bacterial growth in the presence of antimicrobials.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Molecular methods are faster than culture-based methods, and can detect the presence of ARGs, even in bacteria that are difficult to culture in the lab.[ADDIN EN.CITE <EndNote><Cite><Author>Luby</Author><Year>2016</Year><RecNum>546</RecNum><DisplayText><style face="superscript">[100]</style></DisplayText><record><rec-number>546</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529501413">546</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Luby, Elizabeth</author><author>Ibekwe, A. Mark</author><author>Zilles, Julie</author><author>Pruden, Amy</author></authors></contributors><titles><title>Molecular Methods for Assessment of Antibiotic Resistance in Agricultural Ecosystems: Prospects and Challenges</title><secondary-title>Journal of Environmental Quality</secondary-title></titles><periodical><full-title>Journal of Environmental Quality</full-title></periodical><pages>441-453</pages><volume>45</volume><number>2</number><dates><year>2016</year></dates><urls><related-urls><url>http://dx.doi.org/10.2134/jeq2015.07.0367</url></related-urls></urls><electronic-resource-num>10.2134/jeq2015.07.0367</electronic-resource-num><language>English</language></record></Cite></EndNote>] Although presence of the target gene generally classifies a sample as having resistance, it is important to note that detection of the gene is not equivalent to resistance as defined by clinical standards because genes are not always expressed.[ADDIN EN.CITE <EndNote><Cite><Author>CLSI</Author><Year>2018</Year><RecNum>278</RecNum><DisplayText><style face="superscript">[101, 102]</style></DisplayText><record><rec-number>278</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524057866">278</key></foreign-keys><ref-type name="Web Page">12</ref-type><contributors><authors><author>CLSI</author></authors></contributors><titles><title>M100: Performance Standards for Antimicrobial Susceptibility Testing</title></titles><dates><year>2018</year></dates><urls><related-urls><url>https://clsi.org/standards/products/microbiology/documents/m100/</url></related-urls></urls></record></Cite><Cite><Author>Standardization</Author><Year>2006</Year><RecNum>547</RecNum><record><rec-number>547</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529501659">547</key></foreign-keys><ref-

type name="Standard">58</ref-type><contributors><authors><author>International Organization for Standardization</author></authors></contributors><titles><title>ISO 20776-1:2006 Clinical laboratory testing and in vitro diagnostic test systems -- Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices -- Part 1: Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases</title></titles><dates><year>2006</year></dates><urls></urls></record></Cite></EndNote>] Specifically, the fact that an ARG is detected in a sample, or even in a bacterium, does not mean it translates to expressed resistance or organism viability. Therefore, resistance genes are indicators of the genetic potential for resistance, not explicitly resistant bacteria.

Metagenomics

In classical metagenomics, total DNA extracted from an environmental sample is sequenced extensively. Resistance genes in that environmental sample can then be identified based on sequence similarity to known ARGs. This approach has been used to detect genes in a range of human and animal waste samples, including sewage and wastewater,[ADDIN EN.CITE ADDIN EN.CITE.DATA] hospital waste,[ADDIN EN.CITE <EndNote><Cite><Author>Fróes</Author><Year>2016</Year><RecNum>550</RecNum><DisplayText><style face="superscript">[106]</style></DisplayText><record><rec-number>550</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529501950">550</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fróes, Adriana M.</author><author>da Mota, Fábio F.</author><author>Cuadrat, Rafael R. C.</author><author>Dávila, Alberto M. R.</author></authors></contributors><titles><title>Distribution and Classification of Serine β-Lactamases in Brazilian Hospital Sewage and Other Environmental Metagenomes Deposited in Public Databases</title><secondary-title>Frontiers in Microbiology</secondary-title></titles><periodical><full-title>Frontiers in Microbiology</full-title></periodical><pages>1790</pages><volume>7</volume><dates><year>2016</year><pub-dates><date>11/15
05/05/received
10/25/accepted</date></pub-dates></dates><publisher>Frontiers Media S.A.</publisher><isbn>1664-302X</isbn><accession-num>PMC5108929</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5108929/</url></related-urls></urls><electronic-resource-num>10.3389/fmicb.2016.01790</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] animal and human feces,[ADDIN EN.CITE ADDIN EN.CITE.DATA] and in the guts of farm animals and people.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

The main benefit of metagenomic methods is the ability to detect many different resistance and non-resistance genes present in a sample in a single metagenomic-sequencing run (PCR-based methods require a separate test for every specific gene of interest). There are several limitations for metagenomics. These methods are expensive, and quantification is limited to proportions rather than absolute numbers of resistant organisms. Sensitivity can be limited and may vary significantly, because reads for specific genes are only a small proportion of the total number of reads.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Targeted metagenomic approaches may help to address this issue.[ADDIN EN.CITE <EndNote><Cite><Author>Lanza</Author><Year>2018</Year><RecNum>557</RecNum><DisplayText><style face="superscript">[113]</style></DisplayText><record><rec-number>557</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529502625">557</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lanza, Val F.</author><author>Baquero, Fernando</author><author>Martínez, José Luís</author><author>Ramos-Ruíz, Ricardo</author><author>González-Zorn, Bruno</author><author>Andremont, Antoine</author><author>Sánchez-Valenzuela, Antonio</author><author>Ehrlich, Stanislav Dusko</author><author>Kennedy, Sean</author><author>Ruppé, Etienne</author><author>van Schaik, Willem</author><author>Willems, Rob J.</author><author>de la Cruz, Fernando</author><author>Coque, Teresa M.</author></authors></contributors><titles><title>In-depth resistome analysis by targeted metagenomics</title><secondary-title>Microbiome</secondary-title></titles><periodical><full-title>Microbiome</full-title></periodical><pages>11</pages><volume>6</volume><dates><year>2018</year><pub-dates><date>01/15
03/13/received
12/17/accepted</date></pub-dates></dates><pub-location>London</pub-location><publisher>BioMed Central</publisher><isbn>2049-2618</isbn><accession-num>PMC5769438</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5769438/</url></related-urls></urls><electronic-resource-num>10.1186/s40168-017-0387-y</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] Despite the benefits offered by metagenomic strategies, another limitation is that they can only detect known resistance genes (or proteins). This method, like the targeted molecular approaches described above, cannot detect novel ARGs that do not resemble previously identified genes, and might misclassify genes that have acquired activity against new drugs (e.g., the acquisition of quinolone activity by aminoglycoside acetyl transferases).[ADDIN EN.CITE <EndNote><Cite><Author>Robicsek</Author><Year>2005</Year><RecNum>558</RecNum><DisplayText><style face="superscript">[114]</style></DisplayText><record><rec-number>558</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529502734">558</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Robicsek, Ari</author><author>Strahilevitz, Jacob</author><author>Jacoby, George A.</author><author>Macielag, Mark</author><author>Abbanat, Darren</author><author>Hye Park, Chi</author><author>Bush, Karen</author><author>Hooper, David C.</author></authors></contributors><titles><title>Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase</title><secondary-title>Nature Medicine</secondary-title></titles><periodical><full-title>Nature Medicine</full-title></periodical><pages>83</pages><volume>12</volume><dates><year>2005</year><pub-dates><date>12/20/online</date></pub-dates></dates><publisher>Nature Publishing Group</publisher><work-type>Article</work-type><urls><related-urls><url>http://dx.doi.org/10.1038/nm1347</url></related-urls></urls><electronic-resource-num>10.1038/nm1347
https://www.nature.com/articles/nm1347#supplementary-information</electronic-resource-num></record></Cite></EndNote>] At present, only culture-based methods and the functional genomic methods noted below can reliably detect resistance conferred by novel ARGs.

Lastly, labs will need to address consistency and standardization if metagenomics are to be used widely for assessments. Variation in any step of the process can lead to different estimates of ARG abundance.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Moreover, assigning a given resistance to a specific host organism is difficult, particularly for plasmid-borne genes (although cross-linking methods provide a possible solution). This may be problematic for epidemiological investigations. Additionally, the level of taxonomic identification (i.e., family, genus, species, or strain) for bacteria in the sample is limited by the sequence databases used for analysis.

Functional Genomics

Functional genomic approaches can identify novel ARGs, unlike metagenomic strategies.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Here, fragments of genomic DNA from an environmental sample are cloned and expressed in a convenient host, typically *E. coli*. Transformed hosts can then be screened for resistance to an antimicrobial of interest and the resistance gene identified by conventional sequencing. Functional genomic approaches have been used to identify novel genes in a wide variety of environments.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

While functional genomics is a powerful tool for identifying new ARGs, it is not likely to be useful in general surveillance. The time and effort required to process a single sample is substantial, and the use of a single host species (e.g., *E. coli*) limits the number and type of ARGs that can be detected in a given experiment.

Differences between Methods when Testing for Attribution

It is sometimes necessary to track a resistant pathogen, or a resistance gene, to a specific source, such as a hospital or a farm. Such epidemiological investigations require methods with a high degree of resolution, meaning the ability to distinguish between closely related genes or pathogens.

WGS of bacterial isolates is the gold-standard approach for attribution. The entire genome of each organism is sequenced, so WGS represents the upper limit for detecting variation. Even in pathogens with little overall diversity, isolates can be grouped based on a few shared sequence variants, making this a powerful epidemiological approach. WGS is used regularly in epidemiological investigations of foodborne pathogens in North America and Europe. WGS of foodborne pathogens is now routine for the U.S. FDA, U.S. CDC, the Canadian Food Inspection Agency, and the European Centers for Disease Control (ECDC). Similar methods could be readily applied to environmental samples, with the caveat that bacterial isolates are required for standard approaches.

In some situations, technical or financial considerations might prevent WGS from being used. In this case, other techniques may assist in attribution. Multi-locus sequence typing (MLST), for example, involves PCR amplification and sequencing of multiple genes from an isolate, and has a long history in molecular epidemiology.[ADDIN EN.CITE
<EndNote><Cite><Author>Maiden</Author><Year>1998</Year><RecNum>594</RecNum><DisplayText><style face="superscript">[124]</style></DisplayText><record><rec-number>594</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"

timestamp="1530194201">594</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Maiden, Martin C. J.</author><author>Bygraves, Jane A.</author><author>Feil, Edward</author><author>Morelli, Giovanna</author><author>Russell, Joanne E.</author><author>Urwin, Rachel</author><author>Zhang, Qing</author><author>Zhou, Jiaji</author><author>Zurth, Kerstin</author><author>Caugant, Dominique A.</author><author>Feavers, Ian M.</author><author>Achtman, Mark</author><author>Spratt, Brian G.</author></authors></contributors><titles><title>Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms</title><secondary-title>Proceedings of the National Academy of Sciences</secondary-title></titles><periodical><full-title>Proceedings of the National Academy of Sciences</full-title></periodical><pages>3140-3145</pages><volume>95</volume><number>6</number><dates><year>1998</year></dates><urls><related-urls><url>http://www.pnas.org/content/pnas/95/6/3140.full.pdf</url></related-urls></urls><electronic-resource-num>10.1073/pnas.95.6.3140</electronic-resource-num></record></Cite></EndNote>] Similarly, pulse-field gel electrophoresis (PFGE), where isolates are grouped based on patterns of DNA cleavage, can help to establish relationships between strains. MLST, PFGE, and other methods have lower resolutions than WGS, so may not allow for positive attribution. This is particularly a problem in bacterial species or serotypes that harbor low levels of sequence diversity.

Metagenomic data might also be useful for attribution, particularly when a resistant organism is difficult to culture, or when a resistance gene rather than a particular pathogen is the focus of an investigation. While using metagenomic data for attribution has limitations, recent studies suggest metagenomic data do have promise in epidemiology. Proper attribution and tracking of specific ARGs might require targeted sequencing of plasmids, which are often lost during metagenomic assembly.

Standardizing Methods to Monitor the Impact of Mitigation

For culture-based methods, there are already well-formulated standard procedures for measuring antimicrobial susceptibility. Culture-based methods are widely used to monitor the impact of mitigation measures in clinical and agricultural settings, such as the effects of antimicrobial restriction protocols in animals and humans. Molecular typing of cultured isolates, such as MLST or WGS, is increasingly used to provide additional epidemiological data, and standardized methods are available for clinical use. The same approaches could be used to monitor the impact of mitigation methods in environmental samples. Culture-based methods are most appropriate when one or a few specific bacterial species are to be monitored. Generic *E. coli* are often used as an indicator organism for levels of resistance in the overall community.

In other cases, there may be an interest in monitoring the overall pool of ARGs organisms, requiring the use of molecular or metagenomic methods. Currently, there are no widely used standard procedures for monitoring when using molecular or metagenomic methods. PCR-based methods are readily standardized and very common in clinical diagnostics. However, there are no widely accepted PCR-based techniques to detect ARGs in environmental samples. This is likely because it is difficult to develop a method that will work in all (or many) matrices and the lack of consensus around which specific genes should be targeted. As mentioned, metagenomic studies are highly sensitive to variations in protocols, so differences in DNA extraction technique, sequencing platform, and bioinformatics pipeline can have substantial effects on the outcomes of metagenomic analyses. Developing a standardized protocol for metagenomic analysis is challenging at this time due to limited validation of metagenomic methods and the rapidly changing technology. Further work on developing standardized qPCR and metagenomic pipelines, as well as reference materials, will help in culture-independent monitoring.

C. Once environmental waters are contaminated, what evidence exists that this results in the spread of AMR resulting in an increased threat to human health? Does the amount or type of resistant bacteria predict increased risk to human health? How does the interaction between bacteria and antimicrobials affect AMR?

Different studies have detected antimicrobial-resistant bacteria in environmental waters at sites where people could be exposed.[ADDIN EN.CITE
<EndNote><Cite><Author>Huijbers</Author><Year>2015</Year><RecNum>227</RecNum><DisplayText><style face="superscript">[125]</style></DisplayText><record><rec-number>227</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523994248">227</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Huijbers, Patricia M. C.</author><author>Blaak, Hetty</author><author>de Jong, Mart C. M.</author><author>Graat, Elisabeth A. M.</author><author>Vandenbroucke-Grauls, Christina M. J. E.</author><author>de Roda Husman, Ana Maria</author></authors></contributors><titles><title>Role of the Environment in the Transmission of Antimicrobial Resistance to Humans: A Review</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science &

Technology</full-title></periodical><pages>11993-12004</pages><volume>49</volume><number>20</number><dates><year>2015</year><pub-dates><date>2015/10/20</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/acs.est.5b02566</url></related-urls></urls><electronic-resource-num>10.1021/acs.est.5b02566</electronic-resource-num></record></Cite></EndNote>] For example, probable exposure to ESBL-producing *Enterobacteriaceae* was shown for swimmers. Common ways AMR can spread from environmental waters to humans include, for example:

- Recreational water
- Water used for drinking and washing (potable water)
- Consumable fish and bivalves
- Produce contaminated with treated or non-treated surface water
- Urban waters
- Wastewater

Recreational Exposure

In 2003, an estimated 120 million cases of gastrointestinal disease and 50 million cases of respiratory disease were attributed to swimming in or consuming shellfish harvested from coastal environments contaminated with wastewater. The Organisation for Economic Co-operation and Development, an intergovernmental economic organization with 35 member countries, conducted a systematic review on health outcomes associated with exposure to recreational coastal bathing water. The review concluded that, for bathers compared with non-bathers, there is an increased risk of experiencing the following symptoms:[ADDIN EN.CITE <EndNote><Cite><Author>Leonard</Author><Year>2018</Year><RecNum>229</RecNum><DisplayText><style face="superscript">[126]</style></DisplayText><record><rec-number>229</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523994311">229</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Leonard, Anne F. C.</author><author>Singer, Andrew</author><author>Ukoumunne, Obioha C.</author><author>Gaze, William H.</author><author>Garside, Ruth</author></authors></contributors><titles><title>Is it safe to go back into the water? A systematic review and meta-analysis of the risk of acquiring infections from recreational exposure to seawater</title><secondary-title>International Journal of Epidemiology</secondary-title></titles><periodical><full-title>International Journal of Epidemiology</full-title></periodical><pages>dyx281-

dyx281</pages><dates><year>2018</year></dates><isbn>0300-5771</isbn><urls><related-urls><url>http://dx.doi.org/10.1093/ije/dyx281</url></related-urls></urls><electronic-resource-num>10.1093/ije/dyx281</electronic-resource-num></record></Cite></EndNote>]

- Any illness (odds ratio = 1.86; 95% confidence interval: 1.31-2.64; P = 0.001)
- Ear ailments (odds ratio = 2.05; 95% confidence interval: 1.49-2.82; P < 0.001)
- Gastrointestinal ailments (odds ratio = 1.29; 95% confidence interval: 1.12-1.49; P < 0.001)

As the burden of antimicrobial-resistant microbes and ARGs increase in wastewater, there is likely to be an increase in the proportion of antimicrobial-resistant infections. Recreational waters (and associated beach sands) are increasingly recognized as a reservoir of AMR and ARGs, and are probably important to the development of AMR in pathogenic microbes. The following studies evaluated AMR in recreational waters, and highlighted several ARGs and organism types found in fresh and marine waters. However, it is difficult to compare the studies because the geography, ARGs selected for evaluation, sources of waste, and methods to determine resistance are all different from study to study.

Prospective cohort epidemiological studies on three California beaches correlated the detection of a variety of indicators (antimicrobial-resistant bacteria and pathogens) with incidence of gastrointestinal illness.[ADDIN EN.CITE ADDIN EN.CITE.DATA] MRSA was highly associated with gastrointestinal illness. The presence of MRSA was attributed to human sewage and faulty infrastructure. This work highlights that recreational visitors could be exposed to high levels of drug-resistant pathogens if infrastructure is inadequate. A separate study evaluated the prevalence of *S. aureus* and MRSA in ten freshwater beaches in Northeast Ohio.[ADDIN EN.CITE <EndNote><Cite><Author>Thapaliya</Author><Year>2017</Year><RecNum>621</RecNum><DisplayText><style face="superscript">[128]</style></DisplayText><record><rec-number>621</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1540567088">621</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Thapaliya, Dipendra</author><author>Hellwig, Emily J.</author><author>Kadariya, Jhalka</author><author>Grenier, Dylan</author><author>Jefferson, Anne J.</author><author>Dalman, Mark</author><author>Kennedy, Kristen</author><author>DiPerna, Mackenzi</author><author>Orihill, Adrienne</author><author>Taha, Mohammed</author><author>Smith, Tara C.</author></authors></contributors><titles><title>Prevalence and Characterization of Staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus on Public Recreational Beaches in Northeast Ohio</title><secondary-title>GeoHealth</secondary-title></titles><periodical><full-

title>GeoHealth</full-title></periodical><pages>320-332</pages><volume>1</volume><number>10</number><dates><year>2017</year></dates><urls><related-urls><url>https://agupubs.onlinelibrary.wiley.com/doi/abs/10.1002/2017GH000106</url></related-urls></urls><electronic-resource-num>doi:10.1002/2017GH000106</electronic-resource-num></record></Cite></EndNote>] The overall prevalence of *S. aureus* in sand and water samples was 22.8% (64/280). The prevalence of MRSA was 8.2% (23/280). The highest prevalence was observed in summer (45.8%; 55/120) compared to fall (4.2%; 5/120) and spring (10.0%; 4/40). The results of this study indicate *S. aureus*, including MRSA was present in beach sand and freshwater in Northeast Ohio. The high prevalence of *S. aureus* in summer months and the presence of human-associated strains might indicate the possible role of human activity in increasing the prevalence of *S. aureus* in beach water and sand.

A case-control study evaluated the risk factors for community-acquired ESBL-positive urinary tract infections. One of several independent risk factors that the study identified was recreational freshwater swimming within the past year (odd ratio = 2.1; 95% confidence interval: 1.0–4.0).[ADDIN EN.CITE ADDIN EN.CITE.DATA] The study suggests swimming might be a risk factor for intestinal colonization with ESBL-positive *E. coli* and a newly acquired ESBL-producing strain from the water might be the cause for subsequent urinary tract infections. The authors noted that this particular environmental link needed to be substantiated with more evidence. Another study found ESBL-producing *E. coli* in surface waters used for recreation. The site was downstream of poultry farms and municipal wastewater discharge points. The concentration of bacteria suggested that swimmers have a 95% risk of being exposed to ESBL-producing *E. coli* when using these recreational waters.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

More research is needed to evaluate public health effects upon exposure, such as colonization, infection, or horizontal gene transfer. Attempts were made to derive population-level exposure estimates to third generation cephalosporins (3GCs) resistant *E. coli* (3GCREC) during marine recreational water use in England and Wales. Authors estimated the prevalence of the 3GCRECs in coastal recreational waters, combined the data with the *E. coli* density from coastal beaches, and applied the information to ingestion volume estimates for various recreational activities. Together, the data resulted in the mean number of 3GCREC ingested during different water sports. Despite a low prevalence of 3GCREC (0.12%), the authors noted that there is a human exposure risk for water users, which can vary by water sport activity.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Leonard et al.[ADDIN EN.CITE ADDIN EN.CITE.DATA] sequenced pooled *E. coli* isolates recovered from routine bathing water samples taken by the UK Environment Agency in 2016 to assess the relative abundance of ARGs. It was estimated that every bather ingested at least one resistant *E. coli* in 2016, and there were an estimated 2.5 million exposures involving ingestion of at least 100 ARG-positive *E. coli*.

It is important to understand the risk of exposure from contaminated recreational waters. A cross-sectional epidemiological study compared regular surfers with non-surfers to evaluate the association between water exposure and gut colonization by 3GCEC. Results indicated that 6.3% of surfers were colonized by $bla_{\text{CTX-M}}$ bearing *E. coli* compared to 1.5% of non-surfers (risk ratio = 4.09; confidence interval: 1.02-16.4). Bacterial density will increase the risk of exposure, as well as the probability of ingesting a sufficient amount that can either cause an infection or result in colonization. The type of exposure also affects the number of antimicrobial-resistant bacteria ingested, with water sports that include submerging the head resulting in much greater exposure than non-head immersion activities. For example, surfers ingest more than 150 ml of water per session, while swimmers only ingest about 30 ml.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Numerous studies demonstrate that colonization with antimicrobial-resistant bacteria places humans at increased risk of infection (e.g., in healthcare settings, infections are greater when patients are first colonized), but most healthy people will resolve colonization without significant health impact. When colonization first proceeds infection, the time span between colonization and infection may be quite narrow. An intact, mature microbiome in the gastrointestinal tract can help to prevent colonization, but the microbiome can be disrupted by antimicrobials and other environmental exposures. This leaves individuals more susceptible to colonization by antimicrobial resistant bacteria. Particularly susceptible populations include recently hospitalized patients, debilitated patients with chronic illness, and young children.

Even with an intact microbiome, ongoing high-level exposure to environmental antimicrobial-resistant bacteria may result in temporary or persistent colonization. This is likely the case with the healthy surfers and individuals in the community with ongoing exposure. There has been evidence that removing the ongoing exposures will result in slow clearance, which can be seen in healthy travelers who return colonized from settings where there were, presumably, intense environmental exposure (e.g., water, food).[ADDIN EN.CITE
<EndNote><Cite><Author>Ruppé</Author><Year>2018</Year><RecNum>610</RecNum><DisplayText>

<style face="superscript">[130]</style></DisplayText><record><rec-number>610</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1533663807">610</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Ruppé, Etienne</author><author>Andremont, Antoine</author><author>Armand-Lefèvre, Laurence</author></authors></contributors><titles><title>Digestive tract colonization by multidrug-resistant Enterobacteriaceae in travellers: An update</title><secondary-title>Travel Medicine and Infectious Disease</secondary-title></titles><periodical><full-title>Travel Medicine and Infectious Disease</full-title></periodical><pages>28-35</pages><volume>21</volume><keywords><keyword>Travel</keyword><keyword>Antibiotic resistance</keyword><keyword>Intestinal microbiota</keyword><keyword>Enterobacteriaceae</keyword><keyword>Extended-spectrum beta-lactamases</keyword><keyword>Importation</keyword></keywords><dates><year>2018</year><pub-dates><date>2018/01/01/</date></pub-dates></dates><isbn>1477-8939</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S1477893917301953</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.tmaid.2017.11.007</electronic-resource-num></record></Cite></EndNote>] This colonization typically “clears” over several months, but could result in an infection or transmission when coupled with a microbiome-disruptive event, such as antibiotic use.

Potable Water

Coleman et al.[ADDIN EN.CITE <EndNote><Cite><Author>Coleman</Author><Year>2011</Year><RecNum>239</RecNum><DisplayText><style face="superscript">[131]</style></DisplayText><record><rec-number>239</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524056431">239</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Coleman, B. L.</author><author>Salvadori, M. I.</author><author>McGeer, A. J.</author><author>Sibley, K. A.</author><author>Neumann, N. F.</author><author>Bondy, S. J.</author><author>Gutmanis, I. A.</author><author>McEwen, S. A.</author><author>Lavoie, M.</author><author>Strong, D.</author><author>Johnson, I.</author><author>Jamieson, F. B.</author><author>Louie, M.</author></authors></contributors><titles><title>The role of drinking water in the transmission of

antimicrobial-resistant E. coli</title><secondary-title>Epidemiology and Infection</secondary-
title></titles><periodical><full-title>Epidemiology and Infection</full-title></periodical><pages>633-
642</pages><volume>140</volume><number>4</number><edition>06/23</edition><keywords><key
word>Antibiotic resistance</keyword><keyword>enteric
bacteria</keyword><keyword>epidemiology</keyword><keyword>Escherichia
coli</keyword><keyword>water
(safe)</keyword></keywords><dates><year>2011</year></dates><publisher>Cambridge University
Press</publisher><isbn>0950-2688</isbn><urls><related-
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antimicrobialresistant-e-coli/2ACA6F10D8757663C91DACA9337C43F9</url></related-
urls></urls><electronic-resource-num>10.1017/S0950268811001038</electronic-resource-
num><remote-database-name>Cambridge Core</remote-database-name><remote-database-
provider>Cambridge University Press</remote-database-provider></record></Cite></EndNote>]
demonstrated that having antimicrobial-resistant *E. coli* in the home potable water supply was independently associated with colonization. Under conditions with poor water, sanitation, and hygiene, antimicrobial resistance can be present in water intended for human consumption or food production.[
ADDIN EN.CITE
<EndNote><Cite><Author>Walsh</Author><Year>2011</Year><RecNum>132</RecNum><DisplayText>
<style face="superscript">[132]</style></DisplayText><record><rec-number>132</rec-
number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"
timestamp="1523972312">132</key></foreign-keys><ref-type name="Journal Article">17</ref-
type><contributors><authors><author>Walsh, T. R.</author><author>Weeks,
J.</author><author>Livermore, D. M.</author><author>Toleman, M.
A.</author></authors></contributors><auth-address>Department of Infection, Immunity and
Biochemistry, School of Medicine, Cardiff University, Heath Park, Cardiff, UK.
t.r.walsh@uq.edu.au</auth-address><titles><title>Dissemination of NDM-1 positive bacteria in the New
Delhi environment and its implications for human health: an environmental point prevalence
study</title><secondary-title>Lancet Infect Dis</secondary-title></titles><periodical><full-title>Lancet
Infect Dis</full-title></periodical><pages>355-
62</pages><volume>11</volume><number>5</number><keywords><keyword>Bacteria/drug
effects/*enzymology/*genetics</keyword><keyword>Gene Expression Regulation,
Bacterial/physiology</keyword><keyword>Gene Expression Regulation,

Enzymologic/physiology</keyword><keyword>Humans</keyword><keyword>India/epidemiology</keyword><keyword>*Water Microbiology</keyword><keyword>beta-Lactamases/*genetics/metabolism</keyword></keywords><dates><year>2011</year><pub-dates><date>May</date></pub-dates></dates><isbn>1474-4457 (Electronic)
1473-3099 (Linking)</isbn><accession-num>21478057</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/21478057</url></related-urls></urls><electronic-resource-num>10.1016/S1473-3099(11)70059-7</electronic-resource-num></record></Cite></EndNote>] In regions with more hygiene resources, antimicrobial-resistant microbes, ARGs, and antimicrobials have been detected in source waters for drinking water, but contemporary water treatment processes are very effective at removing such contaminants. The WHO Water Safety Plans outlines risk assessment and risk management frameworks for safe drinking water production, including a recommendation to evaluate the effectiveness of management systems.[ADDIN EN.CITE <EndNote><Cite><Author>Davison</Author><Year>2005</Year><RecNum>601</RecNum><DisplayText><style face="superscript">[133]</style></DisplayText><record><rec-number>601</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1531149512">601</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Davison, A., G. Howard, M. Stevens, P. Callan, L. Fewtrell, D. Deere, J. Bartram</author></authors><tertiary-authors><author>World Health Organization</author></tertiary-authors></contributors><titles><title>Water Safety Plans Managing drinking-water quality from catchment to consumer</title></titles><dates><year>2005</year></dates><publisher>World Health Organization</publisher><urls><related-urls><url>http://www.who.int/water_sanitation_health/dwq/wsp170805.pdf</url></related-urls></urls></record></Cite></EndNote>]

Preventing High-risk Exposure

Despite what may be high levels of antimicrobial-resistant bacteria in environmental surface and sub-surface water, measures can be implemented to reduce the spread of AMR from environmental sources.[ADDIN EN.CITE <EndNote><Cite><Author>Walsh</Author><Year>2011</Year><RecNum>132</RecNum><DisplayText><style face="superscript">[132]</style></DisplayText><record><rec-number>132</rec-

number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972312">132</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Walsh, T. R.</author><author>Weeks, J.</author><author>Livermore, D. M.</author><author>Toleman, M. A.</author></authors></contributors><auth-address>Department of Infection, Immunity and Biochemistry, School of Medicine, Cardiff University, Heath Park, Cardiff, UK. t.r.walsh@uq.edu.au</auth-address><titles><title>Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study</title><secondary-title>Lancet Infect Dis</secondary-title></titles><periodical><full-title>Lancet Infect Dis</full-title></periodical><pages>355-62</pages><volume>11</volume><number>5</number><keywords><keyword>Bacteria/drug effects/*enzymology/*genetics</keyword><keyword>Gene Expression Regulation, Bacterial/physiology</keyword><keyword>Gene Expression Regulation, Enzymologic/physiology</keyword><keyword>Humans</keyword><keyword>India/epidemiology</keyword><keyword>*Water Microbiology</keyword><keyword>beta-Lactamases/*genetics/metabolism</keyword></keywords><dates><year>2011</year><pub-dates><date>May</date></pub-dates></dates><isbn>1474-4457 (Electronic)
1473-3099 (Linking)</isbn><accession-num>21478057</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/21478057</url></related-urls></urls><electronic-resource-num>10.1016/S1473-3099(11)70059-7</electronic-resource-num></record></Cite></EndNote>] For example, recreational water might be treated to remove antimicrobial-resistant bacteria, or it might be segregated from other contaminated environmental surface waters. For potable water, finishing treatment plants and well maintained water supply pipe systems would enhance the probability of AMR-free water at the tap; sewage might be kept from fisheries and bivalve seabeds; or relatively uncontaminated water for produce irrigation. Commonly, risk assessment and risk management frameworks are used to protect consumers, such as bathing water profiles, water safety plans, and the Hazard Analysis Critical Control Point (a management system to address food safety). These frameworks should be evaluated to determine if they can prevent amplification and transmission of antimicrobial resistance.

Proximity to Farms

Several studies have found evidence suggesting that a farm to environment to human route of transmission may occur.[ADDIN EN.CITE ADDIN EN.CITE.DATA] For example, one study identified a higher risk for MRSA colonization in people living in close proximity to farms.[ADDIN EN.CITE <EndNote><Cite><Author>Paget</Author><Year>2015</Year><RecNum>619</RecNum><DisplayText><style face="superscript">[137]</style></DisplayText><record><rec-number>619</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1540400837">619</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Paget, John</author><author>Aangenend, Helen</author><author>Kühn, Malte</author><author>Hautvast, Jeannine</author><author>van Oorschot, Desiree</author><author>Olde Loohuis, Alphons</author><author>van der Velden, Koos</author><author>Friedrich, Alexander W.</author><author>Voss, Andreas</author><author>Köck, Robin</author></authors></contributors><titles><title>MRSA Carriage in Community Outpatients: A Cross-Sectional Prevalence Study in a High-Density Livestock Farming Area along the Dutch-German Border</title><secondary-title>PloS one</secondary-title></titles><periodical><full-title>PLoS One</full-title></periodical><pages>e0139589-e0139589</pages><volume>10</volume><number>11</number><dates><year>2015</year></dates><publisher>Public Library of Science</publisher><isbn>1932-6203</isbn><accession-num>26619190</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/26619190</url><url>https://www.ncbi.nlm.nih.gov/pmc/PMC4664395/</url></related-urls></urls><electronic-resource-num>10.1371/journal.pone.0139589</electronic-resource-num><remote-database-name>PubMed</remote-database-name></record></Cite></EndNote>] Strain types found in people living near farms were like the strain types found in animals from the farm and differed from strain types found in people whose exposure was most likely from healthcare. The exposure resulting in human acquisition MRSA types, like those found on the farm, was unknown. Another study found proximity to swine manure application in crop fields and livestock operations to be a risk factor for MRSA infections.[ADDIN EN.CITE <EndNote><Cite><Author>Casey</Author><Year>2013</Year><RecNum>620</RecNum><DisplayText><style face="superscript">[136]</style></DisplayText><record><rec-number>620</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1540566138">620</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>Casey, Joan A.</author><author>Curriero, Frank C.</author><author>Cosgrove, Sara E.</author><author>Nachman, Keeve E.</author><author>Schwartz, Brian S.</author></authors></contributors><titles><title>High-density livestock operations, crop field application of manure, and risk of community-associated methicillin-resistant Staphylococcus aureus infection in Pennsylvania</title><secondary-title>JAMA internal medicine</secondary-title></titles><periodical><full-title>JAMA internal medicine</full-title></periodical><pages>1980-1990</pages><volume>173</volume><number>21</number><dates><year>2013</year></dates><isbn>2168-6114
2168-6106</isbn><accession-num>24043228</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/24043228</url><url>https://www.ncbi.nlm.nih.gov/pmc/PMC4372690/</url></related-urls></urls><electronic-resource-num>10.1001/jamainternmed.2013.10408</electronic-resource-num><remote-database-name>PubMed</remote-database-name></record></Cite></EndNote>] These findings suggest more information is needed to determine the potential contribution of agriculture to environmental AMR and if this occurs, is there a risk to humans.

D. What mitigation methods are effective in preventing contamination of the environment or decreasing the amount of antimicrobial-resistant pathogens in environmental waters? If effective mitigation methods are lacking, what strategies for preventing contamination or reducing bacteria load are most promising?

There are a range of mitigation options for preventing and reducing the amount of antimicrobial-resistant microbes (including pathogenic microbes) in the environment. The same types of mitigation options apply to human and animal wastes, although interventions and technologies used for animal waste streams tend to be more primitive than systems for people. This section primarily focuses on mitigation related to human systems, partly because more information is available. However, technologies are similar to animal systems and mitigation solutions must be holistic, following a One Health approach that combines non-technical and technical solutions.

When considering mitigation methods, it is important to identify the relevant target (e.g., antimicrobial-resistant human pathogens or ARGs). The primary goal is to reduce human exposure to human antimicrobial-resistant pathogens. However, other factors need to be considered for AMR mitigation, such as antimicrobial-resistant commensals, environmental microbes, and phage vectors.

There is debate among environmental AMR scientists about the importance of environmental microbes, phage, and free DNA as explicit drivers of antimicrobial-resistant pathogens in the environment. Focusing detection methods on quantitative measurements of clinically relevant resistance genes may be adequate as these are likely derived from pathogenic bacteria or bacteria that are able to mobilize resistance to human pathogens.

Global and Local Context on Mitigation Approaches

There is growing evidence that suggests antimicrobial-resistant microbes can move rapidly across continents due to tourism and trade.[ADDIN EN.CITE <EndNote><Cite><Author>Zhu</Author><Year>2017</Year><RecNum>316</RecNum><DisplayText><style face="superscript">[138]</style></DisplayText><record><rec-number>316</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524063325">316</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zhu, Yong-Guan</author><author>Gillings, Michael</author><author>Simonet, Pascal</author><author>Stekel, Dov</author><author>Banwart, Steve</author><author>Penuelas, Josep</author></authors></contributors><titles><title>Microbial mass movements</title><secondary-title>Science</secondary-title></titles><periodical><full-title>Science</full-title></periodical><pages>1099-1100</pages><volume>357</volume><number>6356</number><dates><year>2017</year></dates><urls><related-urls><url>http://science.sciencemag.org/content/sci/357/6356/1099.full.pdf</url></related-urls></urls><electronic-resource-num>10.1126/science.aao3007</electronic-resource-num></record></Cite></EndNote>] For example, the amount of class 1 integron genes, an element that can enable bacteria to transmit resistance, is increasing.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Within this global context, possible mitigation methods should be based on the expense and relative efficacy of each option. As mentioned, the mitigation methods for reducing the amount of antimicrobial-resistant bacteria and ARGs in the environment include non-technical and technical options. The applicability of these options depends on available resources and the cultural context of the interventions. No single mitigation method has proven to be successful; however, applying a combination of these methods based on various factors could help to reduce antimicrobial-resistant microbes and ARGs in the environment. In fact, all evidence suggests that stewardship interventions (e.g., reduce unnecessary use of antimicrobials) without parallel technical interventions (e.g., biological waste treatment), or vice versa, will not reduce environmental levels of antimicrobial resistance. This is especially true in 80% of the world where waste treatment functionally does not exist.[ADDIN EN.CITE <EndNote><Cite><Author>Graham</Author><Year>2014</Year><RecNum>136</RecNum><DisplayText><style face="superscript">[16]</style></DisplayText><record><rec-number>136</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972425">136</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Graham, David W.</author><author>Collignon, Peter</author><author>Davies, Julian</author><author>Larsson, D. G. Joakim</author><author>Snape, Jason</author></authors></contributors><titles><title>Underappreciated Role of Regionally Poor Water Quality on Globally Increasing Antibiotic Resistance</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>11746-11747</pages><volume>48</volume><number>20</number><dates><year>2014</year><pub-dates><date>2014/10/21</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/es504206x</url></related-urls></urls><electronic-resource-num>10.1021/es504206x</electronic-resource-num></record></Cite></EndNote>] However, there is limited information on the relative effectiveness of some options in terms of reducing antimicrobial-resistant microbes and ARGs in the environment.

General mitigation options proposed in the literature include social, behavioral, and managerial interventions like improving antimicrobial use, reducing the release of untreated waste directly into the environment,[ADDIN EN.CITE <EndNote><Cite><Author>Ahammad</Author><Year>2014</Year><RecNum>283</RecNum><DisplayText><style face="superscript">[141]</style></DisplayText><record><rec-number>283</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524059059">283</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Ahammad, Z. S.</author><author>Sreekrishnan, T. R.</author><author>Hands, C. L.</author><author>Knapp, C. W.</author><author>Graham, D. W.</author></authors></contributors><titles><title>Increased Waterborne blaNDM-1 Resistance Gene

Abundances Associated with Seasonal Human Pilgrimages to the Upper Ganges River</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>3014-3020</pages><volume>48</volume><number>5</number><dates><year>2014</year><pub-dates><date>2014/03/04</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/es405348h</url></related-urls></urls><electronic-resource-num>10.1021/es405348h</electronic-resource-num></record></Cite></EndNote>] and reducing "problem" pollutant releases at the source that might promote co-selection of resistance (i.e., heavy metals and biocides).[ADDIN EN.CITE <EndNote><Cite><Author>Pal</Author><Year>2015</Year><RecNum>309</RecNum><DisplayText><style face="superscript">[37]</style></DisplayText><record><rec-number>309</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524062677">309</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pal, Chandan</author><author>Bengtsson-Palme, Johan</author><author>Kristiansson, Erik</author><author>Larsson, D. G. Joakim</author></authors></contributors><titles><title>Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential</title><secondary-title>BMC Genomics</secondary-title></titles><periodical><full-title>BMC Genomics</full-title></periodical><pages>964</pages><volume>16</volume><dates><year>2015</year><pub-dates><date>11/17
09/16/received
10/27/accepted</date></pub-dates></dates><pub-location>London</pub-location><publisher>BioMed Central</publisher><isbn>1471-2164</isbn><accession-num>PMC4650350</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4650350/</url></related-urls></urls><electronic-resource-num>10.1186/s12864-015-2153-5</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] General mitigation options also includes implementing or improving local wastewater management, for example:

- Providing or placing toilets (even without treatment) in homes, communities, and strategic locations to reduce open defecation

- Providing "local," decentralized wastewater management options that will delay fresh fecal matter from entering the receiving waters (e.g., portable toilets), or toilets connected to minimal local "treatment" (e.g., septic tanks, soakaways)
- Providing sewer collection systems that carry community and other wastewaters to a centralized treatment facility, which includes primary, secondary (biological), or tertiary treatment
- Providing sewer collection networks that include targeted pre-treatment for wastes from selected critical sources (e.g., hospitals, manufacturing facilities, etc.), which would reduce the burden of antimicrobial resistance on central wastewater treatment systems
- Providing sewage collection and treatment networks, which also provide more stringent treatment or processing of wastewater biosolids
- Providing sewer collection systems with local pre-treatment and centralized community wastewater treatment, but then additional post-tertiary treatment that might ultimately allow for water reuse

The authors propose measures be applied in different variations and combinations depending on existing infrastructure and the scenario. For example, in the least developed low- and middle-income countries first steps to reduce antimicrobial-resistant microbes and ARGs in the environment could be simply increasing access to toilets and improving rural and decentralized wastewater treatment. In more developed countries, layers of wastewater treatment might be needed, especially when water reuse is critical due to scarcity. This could range from tertiary wastewater treatment to advanced water treatment prior to reuse.

Mitigation Options for Reducing Antimicrobial-resistant Bacteria and ARGs in the Environment

There is growing data on the relative effectiveness of different mitigation methods for removing AMR, especially for secondary (biological) and tertiary wastewater treatment. However, there is also considerable contradiction across the literature about the "best" options. Further, there are some mitigation methods, particularly more rudimentary options, like septic tanks and other decentralized options, where almost no data exists on mitigation potential. The following are different technical mitigation options based on what is known or can be achieved, ranging from improving basic sanitation to advanced tertiary wastewater treatment. The options are described, followed by their potential to reduce AMR.

Improve Basic Wastewater Management: Septic Tanks, Soakaways, and related Options

There is a general shortage of affordable and available small-scale wastewater management and treatment options to reduce local AMR exposures. Such mitigation approaches are critical because the transition from no wastewater sanitation systems (e.g., open defecation) to the placement of latrines (a toilet or outhouse) is potentially dramatic.[ADDIN EN.CITE

<EndNote><Cite><Author>Graham</Author><RecNum>602</RecNum><DisplayText><style face="superscript">[142]</style></DisplayText><record><rec-number>602</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1531149883">602</key></foreign-keys><ref-type name="Unpublished Work">34</ref-type><contributors><authors><author>David Graham</author></authors></contributors><titles></titles><dates></dates><publisher>Newcastle University</publisher><urls></urls></record></Cite></EndNote>] This can be further improved by including well-maintained wastewater treatment processes. When local wastes are better contained, then it is easier to direct wastes for treatment, including using local-scale biological wastewater treatment processes. One example of a local-scale option is denitrifying downflow hanging-sponge reactors, which can reduce antimicrobial-resistant bacteria by more than 90% at almost no energy cost.[ADDIN EN.CITE
<EndNote><Cite><Author>Jong</Author><Year>2018</Year><RecNum>581</RecNum><DisplayText><style face="superscript">[143]</style></DisplayText><record><rec-number>581</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529579759">581</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Jong, Mui-Choo</author><author>Su, Jian-Qiang</author><author>Bunce, Joshua T.</author><author>Harwood, Colin R.</author><author>Snape, Jason R.</author><author>Zhu, Yong-Guan</author><author>Graham, David W.</author></authors></contributors><titles><title>Co-optimization of sponge-core bioreactors for removing total nitrogen and antibiotic resistance genes from domestic wastewater</title><secondary-title>Science of The Total Environment</secondary-title></titles><periodical><full-title>Science of The Total Environment</full-title></periodical><pages>1417-1423</pages><volume>634</volume><keywords><keyword>Antibiotic resistance genes</keyword><keyword>Sustainable wastewater treatment</keyword><keyword>Wastewater bypass</keyword><keyword>Denitrification</keyword><keyword>High-throughput qPCR</keyword></keywords><dates><year>2018</year><pub-dates><date>2018/09/01/</date></pub-dates></dates><isbn>0048-9697</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0048969718312014</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.scitotenv.2018.04.044</electronic-resource-num></record></Cite></EndNote>]

However, there is a broad lack of available simple technologies, which is a major gap in AMR mitigation, especially in low- and middle-income countries. This gap is globally relevant because “minimalist” mitigation approaches may be the only option for removing antimicrobial-resistant microbes from wastes in most of the world. Preliminary data hint that septic tanks can reduce antimicrobial-resistant levels by up to 50% if they are well maintained. Therefore, if latrines with septic tanks, soakaways, or similar processes were implemented, then environmental AMR reductions could be as much as 1,000,000 fold (relative to fecal matter) due to reducing open defecation and providing waste containment.[ADDIN EN.CITE <EndNote><Cite><Author>Graham</Author><RecNum>602</RecNum><DisplayText><style face="superscript">[142]</style></DisplayText><record><rec-number>602</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1531149883">602</key></foreign-keys><ref-type name="Unpublished Work">34</ref-type><contributors><authors><author>David Graham</author></authors></contributors><titles></titles><dates></dates><publisher>Newcastle University</publisher><urls></urls></record></Cite></EndNote>] Such reductions could be further enhanced using local-scale technologies, such as denitrifying downflow hanging-sponge. Improving fundamental sanitation is crucial, but long-term maintenance and support is also critical, and is a global challenge in both developed and developing countries.

Conventional Secondary Wastewater Treatment

WWTPs use various treatment steps. Initial screening and primary sewage settling removes inert and biological solids, including antimicrobial-resistant bacteria within the readily settable solids. This is similar to what occurs in minimalist mitigation wastewater treatment options. After primary settling, the technology used in the biological treatment step determines if antimicrobial-resistant microbes are removed or pass untreated. Biological treatment (also called secondary treatment) is intended to remove soluble organic matter (microorganisms grow on that matter, including organisms from the original wastes and organisms enriched in the process). After biological treatment, this mixed microbial community is separated from the liquid stream by secondary settling (or sometimes by filtration). This creates two effluent streams that are processed separately—supernatant liquid effluents and biosolids.

Specific biological treatment processes vary widely in their ability to reduce resistant microbes and ARGs. For example, conventional biological treatment typically removes around 90% of ARGs after primary treatment, with some technologies removing up to 99% or more.[ADDIN EN.CITE

<EndNote><Cite><Author>Graham</Author><RecNum>602</RecNum><DisplayText><style face="superscript">[142]</style></DisplayText><record><rec-number>602</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1531149883">602</key></foreign-keys><ref-type name="Unpublished Work">34</ref-type><contributors><authors><author>David Graham</author></authors></contributors><titles></titles><dates></dates><publisher>Newcastle University</publisher><urls></urls></record></Cite></EndNote>] However, these estimates are for the liquid effluents only. This does not account for resistant microbes and ARGs separated into the biosolids stream. Also, there is some concern about selective agents in the wastewater, such as residual metals and antimicrobials, which might promote elevated horizontal gene transfer between bacteria within biological treatment systems. Although there is some evidence that this occurs, rates of gene transfer in activated sludge appear to be relatively low.[ADDIN EN.CITE <EndNote><Cite><Author>Munck</Author><Year>2015</Year><RecNum>305</RecNum><DisplayText><style face="superscript">[144]</style></DisplayText><record><rec-number>305</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524059747">305</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Munck, Christian</author><author>Albertsen, Mads</author><author>Telke, Amar</author><author>Ellabaan, Mostafa</author><author>Nielsen, Per Halkjær</author><author>Sommer, Morten O. A.</author></authors></contributors><titles><title>Limited dissemination of the wastewater treatment plant core resistome</title><secondary-title>Nature Communications</secondary-title></titles><periodical><full-title>Nature Communications</full-title></periodical><pages>8452</pages><volume>6</volume><dates><year>2015</year><pub-dates><date>09/30/online</date></pub-dates></dates><publisher>The Author(s)</publisher><work-type>Article</work-type><urls><related-urls><url>http://dx.doi.org/10.1038/ncomms9452</url></related-urls></urls><electronic-resource-num>10.1038/ncomms9452
https://www.nature.com/articles/ncomms9452#supplementary-information</electronic-resource-num></record></Cite></EndNote>] More work is needed to determine the extent of resistance transfer within WWTPs.

Growing evidence suggests that a major factor contributing to the global AMR threat is the wide lack of secondary level treatment in most of the world, rather than weaknesses in existing technologies. However, this does not mean that current biological treatment options are perfect. There is evidence that specific types of resistance can be selected for during wastewater processing.[ADDIN EN.CITE <EndNote><Cite><Author>Bengtsson-Palme</Author><Year>2016</Year><RecNum>273</RecNum><DisplayText><style face="superscript">[145]</style></DisplayText><record><rec-number>273</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524070604">273</key><key app="ENWeb" db-id="">0</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bengtsson-Palme, Johan</author><author>Hammarén, Rickard</author><author>Pal, Chandan</author><author>Östman, Marcus</author><author>Björlenius, Berndt</author><author>Flach, Carl-Fredrik</author><author>Fick, Jerker</author><author>Kristiansson, Erik</author><author>Tysklind, Mats</author><author>Larsson, D. G. Joakim</author></authors></contributors><titles><title>Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics</title><secondary-title>Science of The Total Environment</secondary-title></titles><periodical><full-title>Science of The Total Environment</full-title></periodical><pages>697-712</pages><volume>572</volume><keywords><keyword>Antibiotic resistance genes</keyword><keyword>Co-selection</keyword><keyword>Fecal bacteria</keyword><keyword>Microbial ecology</keyword><keyword>Risk assessment</keyword><keyword>Wastewater treatment</keyword></keywords><dates><year>2016</year><pub-dates><date>2016/12/01/</date></pub-dates></dates><isbn>0048-9697</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0048969716314176</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.scitotenv.2016.06.228</electronic-resource-num></record></Cite></EndNote>] There is also growing evidence that a small sub-fraction of antimicrobial-resistant enteric bacteria that enter WWTPs in the wastes, including pathogens, selectively survive the current secondary treatment systems.[ADDIN EN.CITE <EndNote><Cite><Author>Quintela-Baluja</Author><Year>2018</Year><RecNum>142</RecNum><DisplayText><style face="superscript">[14]</style></DisplayText><record><rec-number>142</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972981">142</key></foreign-keys><ref-type name="Thesis">32</ref-type><contributors><authors><author>Quintela-Baluja, M</author></authors></contributors><titles><title>Urban water cycle and antibiotic resistance genes dissemination</title></titles><volume>Doctor of Philosophy</volume><dates><year>2018</year></dates><publisher>Newcastle University</publisher><urls></urls></record></Cite></EndNote>] The reasons for this are not known, and require further investigation.

To address these weaknesses, process modifications and retrofits of existing WWTPs are being developed to improve the ability of existing WWTPs to reduce the release of antimicrobial-resistant microbes and ARGs. For example, sequencing anaerobic-aerobic bioreactors can reduce ARG diversity and abundances in treated effluents by a further 60%.[ADDIN EN.CITE <EndNote><Cite><Author>Christgen</Author><Year>2015</Year><RecNum>292</RecNum><DisplayText><style face="superscript">[146]</style></DisplayText><record><rec-number>292</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524059299">292</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Christgen, Beate</author><author>Yang, Ying</author><author>Ahammad, S. Z.</author><author>Li, Bing</author><author>Rodriquez, D. Catalina</author><author>Zhang, Tong</author><author>Graham, David W.</author></authors></contributors><titles><title>Metagenomics Shows That Low-Energy Anaerobic–Aerobic Treatment Reactors Reduce Antibiotic Resistance Gene Levels from Domestic Wastewater</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>2577-2584</pages><volume>49</volume><number>4</number><dates><year>2015</year><pub-dates><date>2015/02/17</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/es505521w</url></related-urls></urls><electronic-resource-num>10.1021/es505521w</electronic-resource-num></record></Cite></EndNote>] Other technologies, such as membrane-separation processes have shown promising results at removing antimicrobial-resistant microbes, and pre-treating sources prior to releasing into sewers might be effective at removing bacteria capable of horizontal gene transfer prior to entering WWTPs (e.g., from hospital wastewater sources within sewage catchments).

Tertiary Wastewater Treatment

Tertiary treatment options for secondary WWTP effluents include using disinfectants and other oxidants, and various options for filtration. Chlorine disinfection can achieve approximately 99% removal of bacteria when using typical chlorine doses and contact times. However, antimicrobial-resistant bacteria appear slightly less susceptible to chlorination, so higher doses may be needed to further reduce antimicrobial-resistant bacteria. However, higher doses might also generate higher levels of potentially carcinogenic disinfection by-products, which is a concern for potential water reuse.

Ultraviolet (UV) disinfection is an alternative to chlorine because it does not generate disinfection by-products. Doses between 5.0 and ~200 mJ/cm^2 are typically used to inactivate microbes in normal disinfection, and doses between 10 to 20 mJ/cm^2 have been found to inactivate up to 99.9% of the antimicrobial-resistant bacteria. However, ARG measurements indicate only 90-99% removal, even at comparatively higher UV doses. UV treatment is promising, but UV systems are less effective in the presence of greater solid matter, a common problem with wastewater treatment.

Beyond chlorination and UV, tertiary options for reducing bacterial and other loads include ozonation, and other advanced oxidation processes. Ozone is a strong oxidizing agent that has shown promise in destroying bacteria and pathogens, which, in turn, can reduce antimicrobial-resistant bacteria and ARG levels with adequate doses and contact times. However, ozonation is very costly, and evidence suggests that some strains can increase with ozonation, including antimicrobial-resistant *E. coli* and *Staphylococcus* species.[ADDIN EN.CITE <EndNote><Cite><Author>Lüddeke</Author><Year>2015</Year><RecNum>302</RecNum><DisplayText><style face="superscript">[147]</style></DisplayText><record><rec-number>302</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524059688">302</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lüddeke, Frauke</author><author>Heß, Stefanie</author><author>Gallert, Claudia</author><author>Winter, Josef</author><author>Güde, Hans</author><author>Löffler, Herbert</author></authors></contributors><titles><title>Removal of total and antibiotic resistant bacteria in advanced wastewater treatment by ozonation in combination with different filtering techniques</title><secondary-title>Water Research</secondary-title></titles><periodical><full-title>Water Research</full-title></periodical><pages>243-251</pages><volume>69</volume><keywords><keyword>Fecal indicator bacteria</keyword><keyword>Staphylococci</keyword><keyword>Antibiotic resistance</keyword><keyword>Wastewater treatment</keyword><keyword>Ozonation</keyword></keywords><dates><year>2015</year><pub-dates><date>2015/02/01/</date></pub-dates></dates><isbn>0043-1354</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S004313541400788X</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.watres.2014.11.018</electronic-resource-num></record></Cite></EndNote>] Despite these issues, ozonation is a possible tertiary treatment option because it appears to be more effective in killing bacteria than chlorination or UV.

Other tertiary mitigation options include combining disinfectants and other technologies, such as microfiltration, ultrafiltration, nanofiltration, and reverse osmosis. Of these options, membrane-based technologies seem to be the most effective at reducing antimicrobial-resistant bacteria and ARGs. Such technologies can be used in tertiary wastewater treatment or possibly in water reuse, and can be effective against an array of bacteria. However, specific AMR reduction data are limited for antimicrobial-resistant bacteria and ARGs, except with membrane-separation technologies. Further, membrane-based mitigation technologies tend to be more expensive and would be limited to well-resourced applications.

Pre-treatment at Source Prior to Entering the Sewer System

Some wastewater sources to sewers (e.g., hospital wastewater) can have higher antimicrobial-resistant microbes and ARG abundances, or release antimicrobial-resistant microbes that are more susceptible to horizontal gene transfer. Lamba et al.[ADDIN EN.CITE <EndNote><Cite><Author>Lamba</Author><Year>2017</Year><RecNum>215</RecNum><DisplayText><style face="superscript">[12]</style></DisplayText><record><rec-number>215</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523993057">215</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lamba, Manisha</author><author>Graham, David W.</author><author>Ahammad, S. Z.</author></authors></contributors><titles><title>Hospital Wastewater Releases of Carbapenem-Resistance Pathogens and Genes in Urban India</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>13906-13912</pages><volume>51</volume><number>23</number><dates><year>2017</year><pub-dates><date>2017/12/05</date></pub-dates></dates><publisher>American Chemical

Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/acs.est.7b03380</url></related-urls></urls><electronic-resource-num>10.1021/acs.est.7b03380</electronic-resource-num></record></Cite></EndNote>] studied CRE, $\mathit{bla}_{\text{NDM-1}}$, horizontal gene transfer, and fecal indicators in wastewaters from Indian hospitals that had their own WWTPs. Very high levels of CRE and $\mathit{bla}_{\text{NDM-1}}$ were found in the treated hospital effluents; however, qualitative evidence suggests that few of the WWTPs in the study were well managed or suitable for reducing antimicrobial-resistant bacteria.

Although not currently practiced in wastewater treatment in most countries, targeted treatment at critical sources like hospital wastewater might be a valuable strategy for reducing the AMR burden on existing community WWTPs. In fact, this might be a preferred strategy because the cost of treating wastewater is based on the technology used and the volume of waste treated. Source treatment is attractive because treated volumes can be much lower, which means more aggressive and costly technologies might be used at major AMR sources.

Picking a method for source treatment requires an understanding of the microbiological environment, including understanding the resistome. If key sources are identified, cost-effective pre-treatment solutions are possible, which can be coupled with retrofitting existing WWTPs to reduce antimicrobial-resistant bacteria released into the environment, including pathogens.

Wastewater Biosolids Processing, including Animal Manure

Research has shown that 90-95% of ARGs in untreated municipal wastewater are physically removed because they are separated into the wastewater solids. Numerous technologies are available and used, in practice, to reduce the organic content and to inactivate pathogens in residual wastewater solids. Not surprisingly, these technologies are also capable of reducing the quantities of ARGs with varying degrees of efficacy. The U.S. EPA recommends five treatment processes "to significantly reduce pathogens" from biosolids: aerobic and anaerobic digestion, air drying, composting, and lime stabilization. These "processes to significantly reduce pathogens" consistently reduce the density of pathogenic bacteria, viruses, or parasites in mixed sludge from a conventional plant. In addition there are seven "processes to further reduce pathogens"; composting, heat drying, heat treatment, thermophilic aerobic digestion, beta ray irradiation, gamma ray irradiation, and pasteurization. These processes are used to consistently reduce sewage sludge pathogens to below detectable levels at the time the treated sludge is used or disposed. In general, processes to further reduce pathogens can moderately destroy ARGs, whereas processes to significantly reduce pathogens can achieve more rapid and extensive destruction of ARGs.[

ADDIN EN.CITE <EndNote><Cite><RecNum>603</RecNum><DisplayText><style face="superscript">[148]</style></DisplayText><record><rec-number>603</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1531151293">603</key></foreign-keys><ref-type name="Web Page">12</ref-type><contributors></contributors><titles><title>Examples of Equivalent Processes: PFRP and PSRP</title></titles><volume>2018</volume><number>July 7</number><dates></dates><publisher>U.S. EPA</publisher><urls><related-urls><url>https://www.epa.gov/biosolids/examples-equivalent-processes-pfrp-and-psrp</url></related-urls></urls></record></Cite></EndNote>]
These same technologies can also be used to treat animal manure, although this practice is much less common. Instead, animal manure is usually applied directly (with no or minimal treatment) to soils, where ARGs decay at much slower rates. In fact, ARGs can be detected for at least six months at levels greater than pre-manure application,[ADDIN EN.CITE <EndNote><Cite><Author>Marti</Author><Year>2014</Year><RecNum>188</RecNum><DisplayText><style face="superscript">[60]</style></DisplayText><record><rec-number>188</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523992147">188</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Marti, Romain</author><author>Tien, Yuan-Ching</author><author>Murray, Roger</author><author>Scott, Andrew</author><author>Sabourin, Lyne</author><author>Topp, Edward</author></authors></contributors><titles><title>Safely Coupling Livestock and Crop Production Systems: How Rapidly Do Antibiotic Resistance Genes Dissipate in Soil following a Commercial Application of Swine or Dairy Manure?</title><secondary-title>Applied and Environmental Microbiology</secondary-title></titles><periodical><full-title>Applied and Environmental Microbiology</full-title></periodical><pages>3258-3265</pages><volume>80</volume><number>10</number><dates><year>2014</year><pub-dates><date>01/21/received
03/10/accepted</date></pub-dates></dates><pub-location>1752 N St., N.W., Washington, DC</pub-location><publisher>American Society for Microbiology</publisher><isbn>0099-2240
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possible for ARGs to accumulate over time when animal manure is applied to soil more than twice per year.

Treated wastewater solids are also applied to soils as a conditioner or fertilizer. The presence and persistence of ARGs in soil treated with wastewater solids or untreated animal manure can be elevated compared to controls (soil without application of wastewater solids or manure).[ADDIN EN.CITE ADDIN EN.CITE.DATA] However, elevated levels will decline upon reduced antimicrobial use in source humans and animals.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Using the U.S. EPA pathogen reduction processes significantly reduces the presence of ARGs.[ADDIN EN.CITE ADDIN EN.CITE.DATA] A study showed that ARG levels in soils returned to background levels within six months when wastewater solids were treated using the "processes to significantly reduce pathogens," but ARG levels remain elevated when compared to controls when wastewater solids were only treated using processes to further reduce pathogens. This confirms that the "process to significantly reduce pathogens" is most effective in removing AR bacteria.[ADDIN EN.CITE <EndNote><Cite><Author>Burch</Author><Year>2017</Year><RecNum>289</RecNum><DisplayText><style face="superscript">[154]</style></DisplayText><record><rec-number>289</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524059226">289</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burch, Tucker R.</author><author>Sadowsky, Michael J.</author><author>LaPara, Timothy M.</author></authors></contributors><titles><title>Effect of Different Treatment Technologies on the Fate of Antibiotic Resistance Genes and Class 1 Integrons when Residual Municipal Wastewater Solids are Applied to Soil</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>14225-14232</pages><volume>51</volume><number>24</number><dates><year>2017</year><pub-dates><date>2017/12/19</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/acs.est.7b04760</url></related-urls></urls><electronic-resource-num>10.1021/acs.est.7b04760</electronic-resource-num></record></Cite></EndNote>]

Improving the treatment and handling of wastewater solids and animal manure may offer a substantial opportunity for mitigating the spread of ARGs, and can be done by implementing effective processes and technologies for treating wastewater solids, treating animal manure more widely, and applying wastewater solids and animal manure to soils less frequently.

Antimicrobial Manufacturing Waste

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Summary

- Release of active pharmaceutical ingredients (APIs) into the environment may occur when antimicrobials are manufactured without effective control measures in place. The manufacturing process can result in a high amount of antimicrobials in the surrounding environment (e.g., soil, water), which may lead to selecting for antibiotic resistant bacteria
- The selective pressure from antibiotic contamination can result in elevated concentrations of resistant bacteria in environmental waters. We know that humans exposed to recreational waters with high concentrations of resistant bacteria are at an increased risk of colonization and infection.
- It is unclear how significantly manufacturing waste might contaminate the environment, but there is potential for high-level contamination because of the large quantity of antimicrobial waste generated during the production process.
- Understanding the amount of APIs released into the environment or generating an assessment of risk requires access to discharge data. However, most manufacturers do not voluntarily disclose and are not required to report APIs released in wastewater discharges.
- There are no international standards for wastewater limits for antimicrobials.
- Scientific methods to analyze active pharmaceutical ingredients in discharged manufacturing wastes and in aquatic environments exist, but an internationally recognized standard method is needed for comparison of results.
- The Industry Roadmap for Progress on Combating Antimicrobial Resistance and adopted by 13 industry leaders, outline critical steps to reduce the environmental impact from antimicrobial manufacturing. The Access to Medicines Foundation is continuing this effort by working with stakeholders to update the January 2018 Antimicrobial Resistance Benchmarks (AMRB) that include environmental stewardship metrics for release in 2020.

Addressing Knowledge Gaps

Scientific review suggests that the following actions could improve understanding and guide action:

- Develop and validate standardized monitoring methods for testing antimicrobial agent runoff from the manufacturing process
- ~~Develop and validate standardized monitoring methods for testing antimicrobial agent runoff from the manufacturing process. These methods should include standard sampling schemes,~~

~~sample processing, sample analysis, and specify specific antimicrobial degradation products to measure.~~

- Conduct pilot studies to evaluate the feasibility and cost of limiting discharge to discharge targets (i.e., discharge limits) proposed by scientific experts.
- Identify and evaluate incentives (e.g., green procurement) to reduce pharmaceutical manufacturing contaminants in a timely and effective way.
- Identify or develop strategies to limit environmental contamination in countries where antimicrobial manufacturing occurs. Work with industry partners, such as the AMR Industry Alliance, to evaluate strategies.

Background Statement

Antimicrobials can be released into the environment when they are manufactured without effective control measures in place. The amount of antimicrobials released can be very high and can result in increased levels of antimicrobial resistance in the environment. Manufacturing waste can potentially contaminate the environment because of the large amount of antimicrobials used in the production process. It is possible that this environmental contamination can affect human health and measures should be taken to minimize the risk; however, more research is needed to fully understand the risks. Responding to this risk might require:

- Knowledge of antimicrobial manufacturing measures that minimize or eliminate environmental contamination from drug or drug compounds
- Standardized methods to monitor drugs or drug compounds in the environment
- Agreement on acceptable discharges of antimicrobials into the environment
- Improved manufacturing practices

Scientific Issues

A. How and where are antimicrobials manufactured?

Manufacturing Antimicrobials

There are three antimicrobial (specifically antibiotic) manufacturing processes: fermentation, synthetic, and semi-synthetic (Table 2). Most antimicrobials are produced using a fermentation process; approximately 120 drugs currently on the market are produced this way. Antimicrobials are less frequently produced using synthetic or semi-synthetic processes; approximately 50 drugs currently on the market are produced this way.[ADDIN EN.CITE <EndNote><Cite><Author>Lengeler J. W.</Author><Year>2009</Year><RecNum>338</RecNum><DisplayText><style face="superscript">[155]</style></DisplayText><record><rec-number>338</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"

timestamp="1524064722">338</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Lengeler J. W., Drews G., and Schlegel H. G. </author></authors></contributors><titles><title>Biology of the Prokaryotes</title></titles><section>627-651</section><dates><year>2009</year></dates><pub-location>Stuttgart, Germany</pub-location><urls></urls></record></Cite></EndNote>]

In the earliest years, antimicrobials were naturally produced by fungi (i.e., penicillin) or soil bacteria (i.e., streptomycin and tetracycline).[ADDIN EN.CITE <EndNote><Cite><Author>Clardy</Author><Year>2009</Year><RecNum>320</RecNum><DisplayText><style face="superscript">[156]</style></DisplayText><record><rec-number>320</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524063944">320</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Clardy, Jon</author><author>Fischbach, Michael</author><author>Currie, Cameron</author></authors></contributors><titles><title>The natural history of antibiotics</title><secondary-title>Current biology : CB</secondary-title></titles><periodical><full-title>Current biology : CB</full-title></periodical><pages>R437-R441</pages><volume>19</volume><number>11</number><dates><year>2009</year></dates><isbn>0960-9822
1879-0445</isbn><accession-num>PMC2731226</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2731226/</url></related-urls></urls><electronic-resource-num>10.1016/j.cub.2009.04.001</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] Today, microorganisms used in fermentation are often genetically modified to maximize antimicrobial yields. Genetic modification occurs by exposing microorganisms to ultraviolet radiation, x-rays, or other mutagens, which induces (causes) mutations. Gene amplification is another technique used to increase yields. This occurs by inserting copies of genes into a microorganism using plasmids. The genes code for enzymes involved in producing antimicrobials.

There are many ways antimicrobial production waste can enter the environment, including wastewater discharge or solid waste.[ADDIN EN.CITE <EndNote><Cite><Author>Guardabassi</Author><Year>1998</Year><RecNum>328</RecNum><DisplayText><style face="superscript">[157]</style></DisplayText><record><rec-number>328</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524064320">328</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>Guardabassi, Luca</author><author>Petersen, Andreas</author><author>Olsen, John E.</author><author>Dalsgaard, Anders</author></authors></contributors><titles><title>Antibiotic Resistance in Acinetobacter spp. Isolated from Sewers Receiving Waste Effluent from a Hospital and a Pharmaceutical Plant</title><secondary-title>Applied and Environmental Microbiology</secondary-title></titles><periodical><full-title>Applied and Environmental Microbiology</full-title></periodical><pages>3499-3502</pages><volume>64</volume><number>9</number><dates><year>1998</year><pub-dates><date>04/06/received
06/17/accepted</date></pub-dates></dates><publisher>American Society for Microbiology</publisher><isbn>0099-2240
1098-5336</isbn><accession-num>PMC106754</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC106754/</url></related-urls></urls><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] For example, production of 1,000kg of antimicrobial (procaine penicillin G) can produce: [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1976</Year><RecNum>353</RecNum><DisplayText><style face="superscript">[158]</style></DisplayText><record><rec-number>353</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524065348">353</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><authors><author>U.S. EPA</author></authors></contributors><titles><title>Pharmaceutical Industry: Hazardous Waste Generation, Treatment, and Disposal</title></titles><dates><year>1976</year></dates><urls><related-urls><url>https://nepis.epa.gov/Exe/ZyNET.exe/9100QWMX.txt?ZyActionD=ZyDocument&Client=EPA&Index=1976%20Thru%201980&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C76THRU80%5CTXT%5C00000018%5C9100QWMX.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=3</url></related-urls></urls></record></Cite></EndNote>]

- 10,000 kg of wet mycelium

- 35,000 kg of wet biological sludge
- 56,000 liters of waste fermentation broth
- 1,200 liters of waste solvents

Each waste component could potential be a source of antimicrobial contamination during disposal, but the level of active ingredient is likely to vary by antimicrobial and manufacturing process.

Global Production

The supply chain for antimicrobials is complex and global, with many stakeholders involved (Figure 1). Antimicrobial production is highly commercialized because of a heavy global demand. Government authorities play a main role in regulating production.

Each year, antimicrobial production exceeds 100,000 tons worldwide.[ADDIN EN.CITE <EndNote><Cite><Author>Bbosa</Author><Year>2014</Year><RecNum>498</RecNum><DisplayText><style face="superscript">[159]</style></DisplayText><record><rec-number>498</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527623449">498</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Godfrey S. Bbosa</author><author>Norah Mwebaza</author><author>John Odda</author><author>David B. Kyegombe</author><author>Muhammad Ntale</author></authors></contributors><titles><title>Antibiotics/antibacterial drug use, their marketing and promotion during the post-antibiotic golden age and their role in emergence of bacterial resistance</title><secondary-title>Health</secondary-title></titles><periodical><full-title>Health</full-title></periodical><pages>16</pages><volume>Vol.06No.05</volume><dates><year>2014</year></dates><urls><related-urls><url>//www.scirp.org/journal/PaperInformation.aspx?PaperID=43142</url></related-urls></urls><custom7>43142</custom7><electronic-resource-num>10.4236/health.2014.65059</electronic-resource-num></record></Cite></EndNote>] Livestock consumed at least 63,200 tons of antimicrobials in 2010, accounting for nearly 66% of the estimated 100,000 tons of antimicrobials produced.[ADDIN EN.CITE <EndNote><Cite><Author>Resurgence</Author><Year>2015</Year><RecNum>352</RecNum><DisplayText><style face="superscript">[160]</style></DisplayText><record><rec-number>352</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524065293">352</key></foreign-keys><ref-type name="Web Page">12</ref-

type><contributors><authors><author>Third World Resurgence</author></authors></contributors><titles><title>Antibiotic abuse is driving antibiotic resistance </title></titles><dates><year>2015</year></dates><urls><related-urls><url>https://www.twn.my/title2/resurgence/2015/301-302/health1.htm</url></related-urls></urls><custom2>March 19, 2018</custom2></record></Cite></EndNote>] By 2030, some estimates predict an increase of antimicrobial production by at least two-thirds to address the increase in treating animals with antimicrobials and the shift from extensive to intensive farming.[ADDIN EN.CITE <EndNote><Cite><Author>Van Boeckel</Author><Year>2015</Year><RecNum>499</RecNum><DisplayText><style face="superscript">[161]</style></DisplayText><record><rec-number>499</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527623630">499</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Van Boeckel, Thomas P.</author><author>Brower, Charles</author><author>Gilbert, Marius</author><author>Grenfell, Bryan T.</author><author>Levin, Simon A.</author><author>Robinson, Timothy P.</author><author>Teillant, Aude</author><author>Laxminarayan, Ramanan</author></authors></contributors><titles><title>Global trends in antimicrobial use in food animals</title><secondary-title>Proceedings of the National Academy of Sciences of the United States of America</secondary-title></titles><periodical><full-title>Proceedings of the National Academy of Sciences of the United States of America</full-title></periodical><pages>5649-5654</pages><volume>112</volume><number>18</number><dates><year>2015</year><pub-dates><date>03/19</date></pub-dates></dates><publisher>National Academy of Sciences</publisher><isbn>0027-8424
1091-6490</isbn><accession-num>PMC4426470</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4426470/</url></related-urls></urls><electronic-resource-num>10.1073/pnas.1503141112</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>]

Many pharmaceutical producers have outsourced their manufacturing to India and China because of cheaper labor and capital costs. These countries also have weaker environmental protection laws than other countries, according to the Review on Antimicrobial Resistance (2016).[*] Asia is the world's main

* The UK Prime Minister commissioned the Review on Antimicrobial Resistance in July 2014. He asked economist Jim O'Neill to analyze the global problem and propose concrete actions. The U.K. Government and Wellcome Trust jointly supported it.

producer and supplier of active pharmaceutical ingredients (APIs), including antimicrobials. APIs are the biologically active substances within medicines that have an effect on the patient (human or animal).

A Lack of Data to Map API Production

Currently there is little published information available on the amount of APIs produced globally each year, and where this production occurs, as countries do not require this information to be reported. In addition, regulatory requirements for responsible manufacturing vary. For example, the European Medicines Agency's *Guideline on the environmental risk assessment of medicinal products for human use* (2006)[ADDIN EN.CITE <EndNote><Cite><Author>Agency</Author><Year>2006</Year><RecNum>519</RecNum><DisplayText><style face="superscript">[162]</style></DisplayText><record><rec-number>519</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527853879">519</key></foreign-keys><ref-type name="Standard">58</ref-type><contributors><authors><author>European Medicines Agency</author></authors></contributors><titles><title>Environmental risk assessment of medicinal products for human use</title></titles><dates><year>2006</year><pub-dates><date>01 June 2006</date></pub-dates></dates><pub-location>London</pub-location><isbn>EMEA/CHMP/SWP/4447/00 corr 21*</isbn><urls><related-urls><url>http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001004.jsp&mid=WC0b01ac0580a4aa6a</url></related-urls></urls></record></Cite></EndNote>] states that before receiving market authorization, pharmaceutical products should undergo an environmental risk assessment. However, this requirement does not apply to antimicrobials placed on the market before 2006 when the guidelines came into force, and no risk assessments on the development of AMR in the environment are required. In the U.S., regulatory agencies impose limits on environmental waste for domestic manufacturing but not for manufacturing that occurs abroad.

B. To what extent is the environment currently being contaminated with antimicrobials from manufacturing waste and does environmental contamination result in an increase in AMR within the environment?

In terms of impact and potential risks, localized discharges from manufacturing plants might lead to more antimicrobial contamination than the excretion of drugs that people use for therapy (i.e., human waste). Concentrations of APIs that enter wastewater treatment systems from human waste are generally low because the antimicrobials are being used by a small fraction of the population. Additionally, processing treatments reduce antimicrobials in wastewater, although the efficacy of these processes for removal of contaminants vary. As a result, APIs are typically present in post-treatment effluents and receiving river waters at very low (ng/L) concentrations where effective processing treatments are in place.

In contrast, the direct API discharge from manufacturing plants can result in high concentrations of antimicrobials in the surrounding environment.[ADDIN EN.CITE <EndNote><Cite><Author>Larsson</Author><Year>2014</Year><RecNum>337</RecNum><DisplayText><style face="superscript">[163]</style></DisplayText><record><rec-number>337</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524064645">337</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Larsson, D. G. Joakim</author></authors></contributors><titles><title>Pollution from drug manufacturing: review and perspectives</title><secondary-title>Philosophical Transactions of the Royal Society B: Biological Sciences</secondary-title></titles><periodical><full-title>Philosophical Transactions of the Royal Society B: Biological Sciences</full-title></periodical><pages>20130571</pages><volume>369</volume><number>1656</number><dates><year>2014</year></dates><publisher>The Royal Society</publisher><isbn>0962-8436
1471-2970</isbn><accession-num>PMC4213584</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4213584/</url></related-urls></urls><electronic-resource-num>10.1098/rstb.2013.0571</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] In some cases, the concentration of antimicrobials in manufacturing effluents are much higher than in the blood of patients taking these drugs. Larsson et al.[ADDIN EN.CITE <EndNote><Cite><Author>Larsson</Author><Year>2007</Year><RecNum>335</RecNum><DisplayText><style face="superscript">[164]</style></DisplayText><record><rec-number>335</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524064595">335</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Larsson, D. G. Joakim</author><author>de Pedro, Cecilia</author><author>Paxeus, Nicklas</author></authors></contributors><titles><title>Effluent from drug manufactures contains extremely high levels of pharmaceuticals</title><secondary-title>Journal of Hazardous Materials</secondary-title></titles><periodical><full-title>Journal of

Hazardous Materials</full-title></periodical><pages>751-755</pages><volume>148</volume><number>3</number><keywords><keyword>Pharmaceuticals</keyword><keyword>Antibiotics</keyword><keyword>Environment</keyword><keyword>Effluent</keyword><keyword>Toxicity</keyword></keywords><dates><year>2007</year><pub-dates><date>2007/09/30/</date></pub-dates></dates><isbn>0304-3894</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0304389407009909</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.jhazmat.2007.07.008</electronic-resource-num></record></Cite></EndNote>] analyzed a range of APIs in the effluent from a wastewater treatment plant (WWTP) serving about 90 bulk drug manufacturers in India. The study reported ciprofloxacin concentrations between 28-31 mg/L and fluoroquinolones concentrations between 0.15-0.9 mg/L. Lübbert et al.[ADDIN EN.CITE <EndNote><Cite><Author>Lübbert</Author><Year>2017</Year><RecNum>342</RecNum><DisplayText><style face="superscript">[165]</style></DisplayText><record><rec-number>342</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524064873">342</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lübbert, Christoph</author><author>Baars, Christian</author><author>Dayakar, Anil</author><author>Lippmann, Norman</author><author>Rodloff, Arne C.</author><author>Kinzig, Martina</author><author>Sörgel, Fritz</author></authors></contributors><titles><title>Environmental pollution with antimicrobial agents from bulk drug manufacturing industries in Hyderabad, South India, is associated with dissemination of extended-spectrum beta-lactamase and carbapenemase-producing pathogens</title><secondary-title>Infection</secondary-title></titles><periodical><full-title>Infection</full-title></periodical><pages>479-491</pages><volume>45</volume><number>4</number><dates><year>2017</year><pub-dates><date>August 01</date></pub-dates></dates><isbn>1439-0973</isbn><label>Lübbert2017</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1007/s15010-017-1007-2</url></related-urls></urls><electronic-resource-num>10.1007/s15010-017-1007-2</electronic-resource-num></record></Cite></EndNote>] reported concentrations of moxifloxacin, voriconazole, and fluconazole of 0.69, 2.5, and 240 mg/L, respectively, around a manufacturing site in India. Li et al.[ADDIN EN.CITE <EndNote><Cite><Author>Li</Author><Year>2008</Year><RecNum>339</RecNum><DisplayText><style face="superscript">[166]</style></DisplayText><record><rec-number>339</rec-number><foreign-

keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524064773">339</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Li, D.</author><author>Yang, M.</author><author>Hu, J.</author><author>Ren, L.</author><author>Zhang, Y.</author><author>Li, K.</author></authors></contributors><auth-address>State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China.</auth-address><titles><title>Determination and fate of oxytetracycline and related compounds in oxytetracycline production wastewater and the receiving river</title><secondary-title>Environ Toxicol Chem</secondary-title></titles><periodical><full-title>Environ Toxicol Chem</full-title></periodical><pages>80-6</pages><volume>27</volume><number>1</number><keywords><keyword>Chromatography, Liquid</keyword><keyword>Industrial Waste/*analysis</keyword><keyword>Oxytetracycline/*analysis</keyword><keyword>Rivers</keyword><keyword>Spectrometry, Mass, Electrospray Ionization</keyword><keyword>Waste Disposal, Fluid</keyword><keyword>Water Pollutants, Chemical/*analysis</keyword></keywords><dates><year>2008</year><pub-dates><date>Jan</date></pub-dates></dates><isbn>0730-7268 (Print)
0730-7268 (Linking)</isbn><accession-num>18092864</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/18092864</url></related-urls></urls><electronic-resource-num>10.1897/07-080.1</electronic-resource-num></record></Cite></EndNote>] reported a concentration of 20 mg/L of oxytetracycline in treated effluent from a pharmaceutical manufacturing facility in Hebei Province, China. These elevated concentrations of APIs are not only found in manufacturing effluent and river waters. For example, Kristiansson et al.[ADDIN EN.CITE <EndNote><Cite><Author>Kristiansson</Author><Year>2011</Year><RecNum>332</RecNum><DisplayText><style face="superscript">[167]</style></DisplayText><record><rec-number>332</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524064495">332</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kristiansson, Erik</author><author>Fick, Jerker</author><author>Janzon, Anders</author><author>Grabic, Roman</author><author>Rutgersson, Carolin</author><author>Weijdegård, Birgitta</author><author>Söderström, Hanna</author><author>Larsson, D. G. Joakim</author></authors></contributors><titles><title>Pyrosequencing of Antibiotic-Contaminated

River Sediments Reveals High Levels of Resistance and Gene Transfer Elements</title><secondary-title>PLOS ONE</secondary-title></titles><periodical><full-title>PLoS One</full-title></periodical><pages>e17038</pages><volume>6</volume><number>2</number><dates><year>2011</year></dates><publisher>Public Library of Science</publisher><urls><related-urls><url>https://doi.org/10.1371/journal.pone.0017038</url></related-urls></urls><electronic-resource-num>10.1371/journal.pone.0017038</electronic-resource-num></record></Cite></EndNote>] reported ciprofloxacin concentrations of 914 mg per kg organic matter in sediment downstream of an industrial WWTP in India.

Although many studies have reported elevated concentrations of antimicrobials in effluent streams in India and China, there are similar reports from around the globe where antimicrobial manufacturing occurs.[ADDIN EN.CITE <EndNote><Cite><Author>Larsson</Author><Year>2014</Year><RecNum>337</RecNum><DisplayText><style face="superscript">[163]</style></DisplayText><record><rec-number>337</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524064645">337</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Larsson, D. G. Joakim</author></authors></contributors><titles><title>Pollution from drug manufacturing: review and perspectives</title><secondary-title>Philosophical Transactions of the Royal Society B: Biological Sciences</secondary-title></titles><periodical><full-title>Philosophical Transactions of the Royal Society B: Biological Sciences</full-title></periodical><pages>20130571</pages><volume>369</volume><number>1656</number><dates><year>2014</year></dates><publisher>The Royal Society</publisher><isbn>0962-8436
1471-2970</isbn><accession-num>PMC4213584</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4213584/</url></related-urls></urls><electronic-resource-num>10.1098/rstb.2013.0571</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] For example, in Lahore, Pakistan, a study found 49 µg/L of sulfamethoxazole and lower concentrations of several other antimicrobials in waterways downstream of formulation facilities.[ADDIN EN.CITE <EndNote><Cite><Author>Khan</Author><Year>2013</Year><RecNum>331</RecNum><DisplayText><style face="superscript">[168]</style></DisplayText><record><rec-number>331</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524064460">331</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>Khan, Ghazanfar Ali</author><author>Berglund, Björn</author><author>Khan, Kashif Maqbool</author><author>Lindgren, Per-Eric</author><author>Fick, Jerker</author></authors></contributors><titles><title>Occurrence and Abundance of Antibiotics and Resistance Genes in Rivers, Canal and near Drug Formulation Facilities – A Study in Pakistan</title><secondary-title>PLOS ONE</secondary-title></titles><periodical><full-title>PLoS One</full-title></periodical><pages>e62712</pages><volume>8</volume><number>6</number><dates><year>2013</year></dates><publisher>Public Library of Science</publisher><urls><related-urls><url>https://doi.org/10.1371/journal.pone.0062712</url></related-urls></urls><electronic-resource-num>10.1371/journal.pone.0062712</electronic-resource-num></record></Cite></EndNote>] In Korea, concentrations of up to 44 mg/L of lincomycin were found in effluent from a pharmaceutical manufacturer WWTP.[ADDIN EN.CITE <EndNote><Cite><Author>Sim</Author><Year>2011</Year><RecNum>349</RecNum><DisplayText><style face="superscript">[169]</style></DisplayText><record><rec-number>349</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524065179">349</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sim, Won-Jin</author><author>Lee, Ji-Woo</author><author>Lee, Eung-Sun</author><author>Shin, Sun-Kyoung</author><author>Hwang, Seung-Ryul</author><author>Oh, Jeong-Eun</author></authors></contributors><titles><title>Occurrence and distribution of pharmaceuticals in wastewater from households, livestock farms, hospitals and pharmaceutical manufactures</title><secondary-title>Chemosphere</secondary-title></titles><periodical><full-title>Chemosphere</full-title></periodical><pages>179-186</pages><volume>82</volume><number>2</number><keywords><keyword>Pharmaceuticals</keyword><keyword>Wastewater treatment plants</keyword><keyword>Households</keyword><keyword>Livestock farms</keyword><keyword>Hospitals</keyword><keyword>Pharmaceutical manufactures</keyword></keywords><dates><year>2011</year><pub-dates><date>2011/01/01/</date></pub-dates></dates><isbn>0045-6535</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0045653510011690</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.chemosphere.2010.10.026</electronic-resource-

num></record></Cite></EndNote>] In Croatia, concentrations up to 3.8 mg/L of azithromycin were found in effluent from a pharmaceutical manufacturing plant.[ADDIN EN.CITE <EndNote><Cite><Author>Bielen</Author><Year>2017</Year><RecNum>318</RecNum><DisplayText><style face="superscript">[170]</style></DisplayText><record><rec-number>318</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524063864">318</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bielen, Ana</author><author>Šimatović, Ana</author><author>Kosić-Vukšić, Josipa</author><author>Senta, Ivan</author><author>Ahel, Marijan</author><author>Babić, Sanja</author><author>Jurina, Tamara</author><author>González Plaza, Juan José</author><author>Milaković, Milena</author><author>Udiković-Kolić, Nikolina</author></authors></contributors><titles><title>Negative environmental impacts of antibiotic-contaminated effluents from pharmaceutical industries</title><secondary-title>Water Research</secondary-title></titles><periodical><full-title>Water Research</full-title></periodical><pages>79-87</pages><volume>126</volume><keywords><keyword>Antibiotic pollution</keyword><keyword>Drug manufacturing</keyword><keyword>Macrolides</keyword><keyword>Antibiotic resistance</keyword><keyword>Ecotoxicity</keyword></keywords><dates><year>2017</year><pub-dates><date>2017/12/01/</date></pub-dates></dates><isbn>0043-1354</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S004313541730773X</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.watres.2017.09.019</electronic-resource-num></record></Cite></EndNote>]

Although AMR are present in all environments, the amount of ARGs and mobile genetic elements were found to be much higher in environments with high-level antimicrobial contamination.[ADDIN EN.CITE ADDIN EN.CITE.DATA] One study looked at the amount of resistance genes and mobile genetic elements in a recreational lake not contaminated by sewage or industrial waste in Sweden and compared this to levels in a lake in India open to industrial pollution of fluoroquinolone antimicrobials.[ADDIN EN.CITE <EndNote><Cite><Author>Bengtsson-Palme</Author><Year>2014</Year><RecNum>317</RecNum><DisplayText><style face="superscript">[171]</style></DisplayText><record><rec-number>317</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524063784">317</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bengtsson-Palme, Johan</author><author>Boulund, Fredrik</author><author>Fick, Jerker</author><author>Kristiansson, Erik</author><author>Larsson, D. G. Joakim</author></authors></contributors><titles><title>Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India</title><secondary-title>Frontiers in Microbiology</secondary-title></titles><periodical><full-title>Frontiers in Microbiology</full-title></periodical><pages>648</pages><volume>5</volume><dates><year>2014</year><pub-dates><date>12/02
09/28/received
11/07/accepted</date></pub-dates></dates><publisher>Frontiers Media S.A.</publisher><isbn>1664-302X</isbn><accession-num>PMC4251439</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4251439/</url></related-urls></urls><electronic-resource-num>10.3389/fmicb.2014.00648</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] ARGs were 7,000 times more abundant in the Indian lake compared to the Swedish lake. Similarly, more mobile genetic elements were observed in the Indian lake samples when compared to the Swedish lake. In another study, bacterial populations in environments polluted with industrial antimicrobial discharges carried the largest relative abundance and diversity of ARGs when compared to bacterial populations sampled from wastewater sludge, humans, or animals.[ADDIN EN.CITE <EndNote><Cite><Author>Pal</Author><Year>2016</Year><RecNum>384</RecNum><DisplayText><style face="superscript">[172]</style></DisplayText><record><rec-number>384</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524071579">384</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pal, Chandan</author><author>Bengtsson-Palme, Johan</author><author>Kristiansson, Erik</author><author>Larsson, D. G. Joakim</author></authors></contributors><titles><title>The structure and diversity of human, animal and environmental resistomes</title><secondary-title>Microbiome</secondary-title></titles><periodical><full-title>Microbiome</full-title></periodical><pages>54</pages><volume>4</volume><number>1</number><dates><year>2016</year><pub-dates><date>October 07</date></pub-dates></dates><isbn>2049-2618</isbn><label>Pal2016</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1186/s40168-016-0199-5</url></related-urls></urls><electronic-resource-num>10.1186/s40168-016-0199-5</electronic-resource-num></record></Cite></EndNote>]

When bacterial communities are exposed to such high levels of antimicrobials, the resistance levels dramatically increase within the bacteria population, facilitated by mobile genetic elements that can help these resistance genes move to other bacteria. A study in India examined the resistance profiles of 93 pathogenic and non-pathogenic environmental bacterial strains. These strains were from a WWTP receiving antimicrobial manufacturing effluents. Eighty-six percent of these strains were resistant to 20 or more antimicrobials. In addition, 95% of these strains had at least one mobile genetic element.[ADDIN EN.CITE <EndNote><Cite><Author>Marathe</Author><Year>2013</Year><RecNum>343</RecNum><DisplayText><style face="superscript">[173]</style></DisplayText><record><rec-number>343</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524064908">343</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Marathe, Nachiket P.</author><author>Regina, Viduthalai R.</author><author>Walujkar, Sandeep A.</author><author>Charan, Shakti Singh</author><author>Moore, Edward R. B.</author><author>Larsson, D. G. Joakim</author><author>Shouche, Yogesh S.</author></authors></contributors><titles><title>A Treatment Plant Receiving Waste Water from Multiple Bulk Drug Manufacturers Is a Reservoir for Highly Multi-Drug Resistant Integron-Bearing Bacteria</title><secondary-title>PLOS ONE</secondary-title></titles><periodical><full-title>PLoS One</full-title></periodical><pages>e77310</pages><volume>8</volume><number>10</number><dates><year>2013</year></dates><publisher>Public Library of Science</publisher><urls><related-urls><url>https://doi.org/10.1371/journal.pone.0077310</url></related-urls></urls><electronic-resource-num>10.1371/journal.pone.0077310</electronic-resource-num></record></Cite></EndNote>] Another study in China examined the resistance profiles of 341 environmental bacterial strains from a WWTP receiving discharge from an oxytetracycline production plant. The percentage of oxytetracycline resistance strains from the WWTP, river water downstream, and river water upstream to the WWTP was 95%, 86% and 3%, respectively.[ADDIN EN.CITE <EndNote><Cite><Author>Li</Author><Year>2010</Year><RecNum>379</RecNum><DisplayText><style face="superscript">[174]</style></DisplayText><record><rec-number>379</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524071262">379</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Li, Dong</author><author>Yu, Tao</author><author>Zhang, Yu</author><author>Yang, Min</author><author>Li, Zhen</author><author>Liu,

Miaomiao</author><author>Qi, Rong</author></authors></contributors><titles><title>Antibiotic Resistance Characteristics of Environmental Bacteria from an Oxytetracycline Production Wastewater Treatment Plant and the Receiving River</title><secondary-title>Applied and Environmental Microbiology</secondary-title></titles><periodical><full-title>Applied and Environmental Microbiology</full-title></periodical><pages>3444-3451</pages><volume>76</volume><number>11</number><dates><year>2010</year><pub-dates><date>04/16
12/08/received
04/03/accepted</date></pub-dates></dates><publisher>American Society for Microbiology (ASM)</publisher><isbn>0099-2240
1098-5336</isbn><accession-num>PMC2876458</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2876458/</url></related-urls></urls><electronic-resource-num>10.1128/AEM.02964-09</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] Again, mobile genetic elements were commonly found in the strains from the WWTP and river water downstream. Interestingly, the proportion of multi-drug resistant strains from both WWTP and river water downstream were also much higher when compared to river water upstream (96% vs. 28%). Recent studies indicate that a high percentage of multi-drug resistant strains, even in the presence of excess levels of a single antimicrobial, are attributed to mobile genetic elements that contain multiple resistance genes.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Similar studies in India, China and Croatia showed that antimicrobial-resistant bacteria were abundant in rivers at the effluent sites of manufacturing units compared to upstream sites.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Although there is a clear link between manufacturing and elevated levels of antimicrobials in the environment, the lack of discharge data makes it difficult to know the extent of the problem at every site. As described in the 2018 AMR Benchmark report,[ADDIN EN.CITE <EndNote><Cite><Year>2018</Year><RecNum>587</RecNum><DisplayText><style face="superscript">[178]</style></DisplayText><record><rec-number>587</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529605240">587</key></foreign-keys><ref-type name="Report">27</ref-type><contributors></contributors><titles><title>2018 Antimicrobial Resistance Benchmark</title></titles><pages>59</pages><dates><year>2018</year></dates><publisher>Access to Medicine Foundation</publisher><urls><related-urls><url>https://accesstomedicinefoundation.org/publications/2018-antimicrobial-resistance-benchmark/</url></related-urls></urls></record></Cite></EndNote>] companies do not report discharge levels voluntarily. Also, regulatory agencies do not collect such data or set limits. Our knowledge on the impact of environmental exposures on human health is limited, despite reports of high levels of antimicrobial-resistant bacteria and genes in aquatic sources impacted by industrial antimicrobial discharges. We know that human exposure to recreational waters with high levels of resistant bacteria is associated with an increased risk for some infections. A thorough understanding of how of antimicrobials and antimicrobial-resistant microbes can spread in a variety of environmental settings and the impact on human health is urgently needed.

C. Which measures are most important for limiting environmental contamination?

A combination of technical measures and incentives could be implemented to reduce pharmaceutical manufacturing emissions. Both approaches might be required to limit or eliminate environmental contamination from antimicrobial manufacturing in a timely and effective way.

Incentivizing Actions and Regulation

A range of legal, economic, and social incentives can drive reductions in environmental contamination from pharmaceutical manufacturing. These incentives can be implemented through the work of numerous stakeholders, including regulatory authorities, governments, the public, media, international organizations (e.g., WHO), investors, the pharmaceutical industry, academia, and insurance companies.

Antimicrobial procurement needs to consider more than cost and quality; it must consider environmental stewardship across the product lifecycle.

Procurement practices that reward responsible (i.e., green) manufacturing may have the most powerful impact. An example of non-financial incentives come from the Access to Medicine Foundation. This group publishes an independent biennial benchmark report, which shows the pharmaceutical companies adopting stronger practices to limit manufacturing discharge levels. The benchmark report also lists companies that disclose key information about their environmental strategy and supply chain ([HYPERLINK "https://amrbenchmark.org/"]). The Access to Medicine Foundation works with multiple stakeholders, including governments and investors, to ensure recognition and diffusion of best practices across the industry.

Limits for antimicrobial discharges are proposed in the literature (see *Defining Discharge Limits* below). The feasibility of meeting these limits for various antimicrobial manufacturing processes needs to be determined. Agreement upon discharge limits would promote green manufacturing and create equity among manufacturers. Currently, there are no international discharge limits, no transparent monitoring system and little or no regulation in many countries.

Stewardship Actions

An estimated 20-30% of antimicrobials are used inappropriately in human healthcare. [ADDIN EN.CITE <EndNote><Cite><Author>Davies</Author><Year>2018</Year><RecNum>391</RecNum><DisplayText><style face="superscript">[179, 180]</style></DisplayText><record><rec-number>391</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524071855">391</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Davies, Sally C.</author></authors></contributors><titles><title>Reducing inappropriate prescribing of antibiotics in English primary care: evidence and outlook</title><secondary-title>Journal of Antimicrobial Chemotherapy</secondary-title></titles><periodical><full-title>Journal of Antimicrobial Chemotherapy</full-title></periodical><pages>833-834</pages><volume>73</volume><number>4</number><dates><year>2018</year></dates><isbn>0305-7453</isbn><urls><related-urls><url>http://dx.doi.org/10.1093/jac/dkx535</url></related-urls></urls><electronic-resource-num>10.1093/jac/dkx535</electronic-resource-num></record></Cite><Cite><Author>O'Neill</Author><Year>2016</Year><RecNum>392</RecNum><record><rec-number>392</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524074069">392</key></foreign-keys><ref-type name="Web Page">12</ref-type><contributors><authors><author>O'Neill, J., & The Review on Antimicrobial Resistance. </author></authors></contributors><titles><title>Tackling drug-resistant infections globally: Final report and recommendations.</title></titles><dates><year>2016</year></dates><urls><related-urls><url>https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf</url></related-urls></urls></record></Cite></EndNote>] Effective antimicrobial stewardship (e.g., better prescribing practices and appropriate use of diagnostics) can reduce antimicrobial use, reduce the need for manufacturing, and thereby reduce the environmental impacts from manufacturing. Stewardship can help, but this is only a partial solution for reducing environmental loads since the amount of antimicrobials used is still very high and will continue to increase given demand in low- and middle-income countries where access to antimicrobials is still limited.

Technical Actions

There is growing commitment by pharmaceutical companies to implement responsible manufacturing practices. More than 100 companies signed the Davos Declaration on combating antimicrobial resistance in 2016, which required its signatories to "support measures to reduce environmental pollution from antimicrobials." Another example of supply chain action is the Industry Roadmap for Progress on Combating Antimicrobial Resistance, published in 2016 by thirteen pharmaceutical firms, including many of the largest research-oriented companies. Signatories agreed to a plan to reduce the environmental impact from production of antimicrobials by:

- Reviewing manufacturing and supply chains to assess good practice
- Establishing a common framework for managing antimicrobial discharge, building on existing work such as the Pharmaceutical Supply Chain Initiative, and starting to apply it across manufacturing and supply chains by 2018
- Working with stakeholders to develop a practical mechanism to transparently demonstrate that supply chains meet the standards in the framework
- Working with independent technical experts to establish science-driven, risk-based targets for discharge concentrations for antimicrobials and good practice methods to reduce environmental impact of manufacturing discharges by 2020

Industry is also beginning to respond to the risk posed by AMR waste in manufacturing. In January 2018, the AMR Industry Alliance generated a framework for assessing environmental impact from manufacturing ([HYPERLINK "https://www.amrindustryalliance.org/why-the-amr-industry-alliance/"]). The Antibiotic Manufacturing Framework provides a methodology and set of minimum requirements needed to conduct a site risk evaluation of both macro and micro controls in our supply chains.

A general manufacturing practice described for mitigating manufacturing waste is improving the efficiency of manufacturing processes or batch reactor washings to capture and treat wastes before discharge. Standard wastewater treatment technologies have some ability to treat or remove APIs, but removal rates can vary. Manufacturing waste is made up of a complex mixture of different APIs. The mixture depends on the facility, which might produce a range of different drugs. The APIs at any one facility would be mixed with impurities, solvents, buffers, biocides, catalysts, metals, and potentially microorganisms.

Several methods have been described in the literature for handling hazardous pharmaceutical manufacturing waste, with incineration being the most complete method. Innovative methods for the reduction and potential elimination of the antimicrobial properties of pharmaceutical wastewater include the following options:

- Incineration can be effective in eliminating all antimicrobial activity. While it is the most effective treatment, it is likely the most energy-intensive method.
- Microbiological treatment includes the aerobic or anaerobic decomposition of organic components in the waste stream. Where applied, this can be very effective, but potentially incomplete because there are lower-limit thresholds, which could limit its success. Highly toxic components of the waste stream that kill microorganisms can decrease the effectiveness of treatment.
- Enzymatic treatment uses specific enzymes that degrade chemicals in the waste stream. This method does not require live microorganisms, so toxicity issues are less of a concern. It also has a low risk of contaminating the downstream environment because the enzymes will naturally degrade, unlike microbiological treatments.
- Chemical treatment chemically decomposes organic components within a waste stream using an acid base, Fenton oxidation (using free radicals to oxidize a compound), ozone, or chlorine. The waste stream would likely require neutralization and secondary treatment to address the dissolved organic load.
- Adsorption allows for the removal of organic compounds from the waste stream by partitioning them from the aquatic phase to a solid, such as activated carbon. This method can be effective for a wide range of chemicals, but it can also be expensive.
- Photocatalysis uses a specialized piece of equipment called a photoreactor to generate light and free radicals, which treats the waste.
- UV light is a method that replicates the effective UV light emitted by the sun, which degrades many environmental pollutants. A wastewater treatment facility can replicate the sun's ability to degrade chemicals and kill microorganisms.
- Electrochemical degradation is an effective method that oxidizes organic compounds in wastewater. This method is followed by secondary treatments like UV and chemical treatment.

Some of these processes can generate new waste concerns. For example, removing antimicrobials by adsorption creates additional solid wastes, which might require special techniques for disposal. Additionally, degradation techniques requires careful monitoring of conditions and understanding what transformation products (e.g., metabolites) with antimicrobial activity could form during the process. Biological treatment to metabolize APIs can select for antimicrobial-resistant bacteria, which would enter the environment if there were no additional treatment.

Most treatment strategies focus on antimicrobial-containing liquid waste, but solid waste can also be contaminated. For example, the fermentation manufacturing process produces mycelial mats with antimicrobial residues. In some cases, this waste is used as feed on animal farms. This practice may increase the risk for selection of resistance in the animals and their environment if active antimicrobial agent is present in the mat.

D. What is the economic impact of implementing known measures to prevent environmental contamination?

Selecting the most economical route to treat API manufacturing wastewater with antimicrobial activity depends on the following factors:

- Type of compounds to be eliminated
- Accepted level of antimicrobials in the environment
- Type of technology required for treatment
- Volume of the product and waste stream
- Manufacturing location

The cost of the treatment largely depends on the accepted level of antimicrobials after the treatment. Discussion on the acceptable levels of antimicrobials in the receiving environment is ongoing, and limits have been proposed in the literature.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Companies that responsibly produce antimicrobials set their own limits, mainly based on ecotoxicology data or on cellular bioassays. However, these limits do not predict acceptable levels to minimize the risk of developing antimicrobial resistance. One of the actions of the AMR Industry Alliance manufacturing workgroup is to set science-driven, risk-based targets.

In general, biological treatment is the most economical method for treating waste. However, it is possible that a population of microorganisms with the ability to degrade antimicrobial compounds could develop, and, as a result, carry ARGs. Proper handling of surplus sludge and effluent treatment is therefore required. It is also important to recognize that the microorganisms can be lost if the waste stream becomes too toxic. Compounds in the waste stream that could kill the microorganisms must be removed using another treatment method prior to microbiological treatment (e.g., advanced oxidation). It is also likely that the effluent will contain compounds with antimicrobial activity, requiring additional treatment (e.g., carbon treatment).

Incineration is the best method for waste streams with high amounts of organic solvents or other organic compounds. Waste streams with high levels of inorganic material (mainly salts) are usually treated with a multi-step evaporation system, and the antimicrobial compounds in the waste stream might be eliminated during this process. Otherwise, the waste stream needs to be treated prior to the process. The water coming from the incineration unit should be treated microbiologically, and the solids disposed of in line with local regulations, which normally involves dispensing to a landfill.

In many cases, operational costs can be reduced by investing in advanced equipment for treatment. The total cost (defined as the cost of depreciation of the investment and operational cost) of making sure that the antimicrobial level does not exceed the predicted no-effect concentrations value of the antimicrobial in the receiving environment is estimated at 15% of the API or intermediates cost (unpublished estimate from industry authors). A peer-reviewed published economic analysis is needed.

E. Is a standard method for measuring environmental contamination established?

Lack of Standardized Methods and Regulations for Monitoring Antimicrobial Manufacturing Wastes

Wastewater discharges have different characteristics and contaminant concentrations depending on the type of production process. The main chemicals in these effluents are solvents, detergents, disinfectants, and pharmaceutical products, all of which are potentially ecotoxic (toxic to the environment). There are standard methods for monitoring volatile organic compounds (e.g., EPA method 1671[ADDIN EN.CITE <EndNote><Cite><Year>1998</Year><RecNum>524</RecNum><DisplayText><style face="superscript">[182]</style></DisplayText><record><rec-number>524</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527868625">524</key></foreign-keys><ref-type name="Standard">58</ref-type><contributors></contributors><titles><title>Method 1671, Revision A: Volatile Organic Compounds Specific to the Pharmaceutical Manufacturing Industry by GC/FID</title></titles><dates><year>1998</year></dates><publisher>U.S. Environmental Protection Agency</publisher><urls><related-urls><url>https://www.epa.gov/sites/production/files/2015-09/documents/method_1671a_1998.pdf</url></related-urls></urls></record></Cite></EndNote>]) and other water-soluble organic compounds such as formaldehyde, isobutyraldehyde, and furfural (e.g., EPA method 1667[ADDIN EN.CITE <EndNote><Cite><Year>1998</Year><RecNum>525</RecNum><DisplayText><style face="superscript">[183]</style></DisplayText><record><rec-number>525</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527868703">525</key></foreign-keys><ref-type name="Standard">58</ref-type><contributors></contributors><titles><title>Method 1667, Revision A: Formaldehyde,

Isobutyraldehyde, and Furfural by Derivatization Followed by High Performance Liquid Chromatography</title></titles><dates><year>1998</year></dates><publisher>U.S. Environmental Protection Agency</publisher><urls><related-urls><url>https://www.epa.gov/sites/production/files/2015-09/documents/method_1667a_1998.pdf</url></related-urls></urls></record></Cite></EndNote>]). However, there are no standard methods to analyze API residues or their transformation products that might form during wastewater treatment. Not having standard methods for API analysis in manufacturing wastes is an important gap when it comes to investigating the sources and mechanisms of antimicrobial resistance in the environment.

Manufacturers are not required to report the amount of APIs released in wastewater discharges, even though it is considered an important driver of AMR development and growth. Due to the polar nature and low volatility of antimicrobials, analyzing these compounds in environmental and biological samples is commonly done using liquid chromatography (LC) coupled with mass spectrometry (LC-MS) detection. This provides a high degree of selectivity and sensitivity. However, the accuracy of LC-MS analysis can significantly suffer from signal suppression or signal enhancement because co-extracted components in the sample matrix interfere with the chromatographic separation and ionization process in LC-MS.

The amount the matrix affects the signal intensities of target molecules varies greatly, and depends on the type of the molecules and the composition of the matrix inferences (e.g., humic acids, proteins, phospholipids). The most frequently used method for antimicrobial detection involves an LC with a triple quadrupole MS operated under the selected reaction monitoring mode, resulting in a selective tandem MS analysis (LC-MS/MS).[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Advances in instrumentation have resulted in faster and more selective analysis of multiple antimicrobial classes in aqueous samples using ultra-high pressure LC coupled with hybrid quadrupole-linear ion trap MS detection systems.[ADDIN EN.CITE <EndNote><Cite><Author>Gros</Author><Year>2013</Year><RecNum>405</RecNum><DisplayText><style face="superscript">[189]</style></DisplayText><record><rec-number>405</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524075412">405</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gros, Meritxell</author><author>Rodríguez-Mozaz, Sara</author><author>Barceló, Damià</author></authors></contributors><titles><title>Rapid analysis of multiclass antibiotic residues and some of their metabolites in hospital, urban wastewater and river

water by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry</title><secondary-title>Journal of Chromatography A</secondary-title></titles><periodical><full-title>Journal of Chromatography A</full-title></periodical><pages>173-188</pages><volume>1292</volume><keywords><keyword>Antibiotics</keyword><keyword>Ultra-high-performance liquid chromatography</keyword><keyword>Quadrupole-linear ion trap</keyword><keyword>Multi-residue analytical method</keyword><keyword>Analysis of hospital and urban wastewater</keyword><keyword>Analysis of river water</keyword></keywords><dates><year>2013</year><pub-dates><date>2013/05/31/</date></pub-dates></dates><isbn>0021-9673</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S002196731300037X</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.chroma.2012.12.072</electronic-resource-num></record></Cite></EndNote>] The LC-MS methods are very sensitive, with method quantification limits reaching sub-ppt levels (1-100 ng/L), depending on the type of antimicrobials and the complexity of sample matrices. These methods allow for multi-residue analysis. For example, 100 compounds or more can be analyzed within a single short (e.g., 30 minute) analytical run. These analytical runs could potentially include all key antimicrobials, their metabolites, transformation products, and other co-selecting agents such as biocides.

While less common, using gas chromatography (GC) with MS has also been reported (GC-MS). [ADDIN EN.CITE ADDIN EN.CITE.DATA] Using GC-MS is limited to antimicrobials that can be derivatized (chemically changed) to volatile forms. Most analytical laboratories within pharmaceutical and water sectors own or have access to accredited labs with LC-MS or GC-MS capability.

There are published methods for antimicrobial analysis, which usually provide robust validation data to make sure the results can be accurately reproduced. However, these methods are not standard and vary from one laboratory to another. Most data on antimicrobials in aquatic environments are from surface waters receiving discharges from municipal and hospital wastes or from agricultural run-off. In addition, most data result from localized research projects, usually supported by national funding agencies or research foundations. It is difficult, if not impossible, to find data on the amount of antimicrobials in manufacturing wastes at a national and global level because there are no government regulations for antimicrobial manufacturers to provide information on the residual concentrations of antimicrobials, their metabolites, and degradation products. There is a need for greater data collection on antimicrobial concentrations in manufacturing wastes, using standardized methods that are robust, comprehensive, and fit for purpose.

Challenges and Limitations of Current Analytical Methods for Antimicrobials

As mentioned, analyzing antimicrobials in environmental samples using LC-MS is subject to a variety of interferences from matrix components (e.g., high concentration of salts, dissolved organic compounds, proteins, and fatty acids). These components can lead to false-positive and false-negative detections. In fact, measuring antimicrobials in manufacturing wastes might be prone to errors because of high concentrations of precursors (upstream component) of active pharmaceutical ingredients, fermentation by-products, or side-products of chemical synthesis. Additional challenges include poor extraction recoveries, ionization suppression in LC-MS, and unpredictable matrix effects. These are common challenges for antimicrobial environmental analysis, and not limited to analyzing manufacturing waste.[ADDIN EN.CITE <EndNote><Cite><Author>Aga</Author><Year>2016</Year><RecNum>402</RecNum><DisplayText><style face="superscript">[191]</style></DisplayText><record><rec-number>402</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524075267">402</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Aga, Diana S.</author><author>Lenczewski, Melissa</author><author>Snow, Daniel</author><author>Muurinen, Johanna</author><author>Sallach, J. Brett</author><author>Wallace, Joshua S.</author></authors></contributors><titles><title>Challenges in the Measurement of Antibiotics and in Evaluating Their Impacts in Agroecosystems: A Critical Review</title><secondary-title>Journal of Environmental Quality</secondary-title></titles><periodical><full-title>Journal of Environmental Quality</full-title></periodical><pages>407-419</pages><volume>45</volume><number>2</number><dates><year>2016</year></dates><urls><related-urls><url>http://dx.doi.org/10.2134/jeq2015.07.0393</url></related-urls></urls><electronic-resource-num>10.2134/jeq2015.07.0393</electronic-resource-num><language>English</language></record></Cite></EndNote>] Therefore, it is critical to use isotopically labeled analogues of antimicrobials as surrogates during the analysis of manufacturing wastewater to compensate for the variability in the extraction recoveries and matrix effects. Unfortunately, not all antimicrobials have commercially available labeled analogues, in which case an internal standard structurally related to the target antimicrobials should be used as a surrogate to account for losses during sample preparation and measurement. In addition, performance criteria should be established for the LC-MS methods. Examples of such criteria include setting acceptable variability in ion measurements or acceptable retention time shifts in the chromatograms. Finally, the effect of sample storage and sample preparation on the antimicrobial stability should be evaluated. It is not known if the storage temperature, storage length, or chemical additives (e.g., acidification of samples) used prior for filtration or sample extraction will affect the integrity of the analytes.

The concentrations of antimicrobials in surface waters receiving discharges from municipal WWTP effluents are typically found at low concentrations (below μg/L levels), and therefore require extensive sample preparation and concentration. Solid phase extraction (i.e., a process for separation of a compound from a mixture) is the preferred method to extract antimicrobials from liquid matrices, such as river water and wastewater.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Generally, solid phase extraction recoveries done for target antimicrobials ranged from 50 to more than 100%. Low recoveries might be from highly polar antimicrobials with low sorption to the solid phase extraction cartridge. Because the concentrations of antimicrobials in manufacturing wastes are expected to be high (at mg/L levels), it may be possible to perform a "dilute-and-shoot" analysis, where no sample clean up or concentration is performed, eliminating the potential to lose some analytes during solid phase extraction. In a "dilute-and-shoot" approach, a 10-fold or a 100-fold dilution of sample is required prior to injection, making it ideal for high-throughput analysis of antimicrobials in manufacturing wastes. However, before implementing a "dilute-and-shoot" method, it is critical to establish the target quantification levels for the antimicrobials and other analytes in the manufacturing waste. This is necessary to determine if the method quantification limit is sufficient to detect the target concentrations. However, because there are no regulations on the allowable maximum contaminant levels of API residues in the discharged manufacturing wastes, it is not currently possible to recommend the use of "dilute-and-shoot" method as an acceptable cost-effective alternative to the time-consuming solid phase extraction procedures used in traditional methods.

Because some fraction of antimicrobials can sorb in the sediments of receiving waters, or in the biosolids of fermentation broths from the manufacturing wastes, it is also important to determine the concentrations of antimicrobials in solid samples. There are different techniques to extract antimicrobials from solids (suspended particulate matter, sediments, and biota). These techniques range from simple sonication of the solid samples with organic solvents to using accelerated solvent extraction and microwave assisted extraction.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Extracting antimicrobials from solid matrices is difficult, which is why many large monitoring studies focus only on liquid phase. The emphasis on liquid phases has contributed to a gap in knowledge about how antimicrobials cycle in the environment. Future monitoring strategies should consider solid matrices, including suspended particulate matter, sediments, and biota.

The biggest limitation of the current analytical approaches is that they are limited to analyzing a few known target analytes. For example, only the active pharmaceutical ingredients or the parent antimicrobials are commonly included in the analytical method. This means that potential transformation products formed during treatment or disposal in the environment are not considered. Some classes of antimicrobials are unstable in the environment and form transformation products that might still be biologically active. For example, tetracyclines are known to epimerize or hydrolyze,[ADDIN EN.CITE
<EndNote><Cite><Author>Aga</Author><Year>2005</Year><RecNum>394</RecNum><DisplayText><style face="superscript">[194]</style></DisplayText><record><rec-number>394</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524074231">394</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Aga, D. S.</author><author>O'Connor, S.</author><author>Ensley, S.</author><author>Payero, J. O.</author><author>Snow, D.</author><author>Tarkalson, D.</author></authors></contributors><auth-address>Chemistry Department, University at Buffalo, Buffalo, New York, 14260, USA. dianaaga@buffalo.edu</auth-address><titles><title>Determination of the persistence of tetracycline antibiotics and their degradates in manure-amended soil using enzyme-linked immunosorbent assay and liquid chromatography-mass spectrometry</title><secondary-title>J Agric Food Chem</secondary-title></titles><periodical><full-title>J Agric Food Chem</full-title></periodical><pages>7165-71</pages><volume>53</volume><number>18</number><keywords><keyword>Anti-Bacterial Agents/*analysis/*chemistry</keyword><keyword>Chromatography, Liquid</keyword><keyword>Enzyme-Linked Immunosorbent Assay</keyword><keyword>Manure/*analysis</keyword><keyword>Mass Spectrometry</keyword><keyword>Oxytetracycline/analysis/chemistry</keyword><keyword>Soil/*analysis</keyword><keyword>Tetracyclines/*analysis/*chemistry</keyword></keywords><dates><year>2005</year><pub-dates><date>Sep 7</date></pub-dates></dates><isbn>0021-8561 (Print)
0021-8561 (Linking)</isbn><accession-num>16131125</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/16131125</url></related-urls></urls><electronic-

resource-num>10.1021/jf050415+</electronic-resource-num></record></Cite></EndNote>] or form photodegradation products that retain the conjugated tetracycline rings[ADDIN EN.CITE <EndNote><Cite><Author>Eichhorn</Author><Year>2004</Year><RecNum>404</RecNum><DisplayText><style face="superscript">[195]</style></DisplayText><record><rec-number>404</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524075366">404</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Eichhorn, Peter</author><author>Aga, Diana S.</author></authors></contributors><titles><title>Identification of a Photooxygenation Product of Chlortetracycline in Hog Lagoons Using LC/ESI-Ion Trap-MS and LC/ESI-Time-of-Flight-MS</title><secondary-title>Analytical Chemistry</secondary-title></titles><periodical><full-title>Analytical Chemistry</full-title></periodical><pages>6002-6011</pages><volume>76</volume><number>20</number><dates><year>2004</year><pub-dates><date>2004/10/01</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0003-2700</isbn><urls><related-urls><url>https://doi.org/10.1021/ac0494127</url></related-urls></urls><electronic-resource-num>10.1021/ac0494127</electronic-resource-num></record></Cite></EndNote>] suggesting that these transformation products are still biologically active. In addition, antimicrobials in the β-lactam family (e.g., cephalosporins and penicillins) are generally unstable because of the susceptibility of the β-lactam bond to hydrolysis. API transformation products may be present in the environment at higher levels than their parent compounds. [ADDIN EN.CITE ADDIN EN.CITE.DATA] This is one reason why it is important to monitor both API and API transformation products in manufacturing wastes.

Recently, an increasing number of publications reported using high-resolution MS for environmental monitoring in an attempt to move away from target-driven analysis. Liquid chromatography coupled with high-resolution MS, such as quadrupole time-of-flight MS and Orbitrap^{TM} MS, allow for target analysis to be done alongside non-target screening, and, more importantly, it offers the possibility for retrospective analysis. Storing long-term data sets that allow retrospective analysis could revolutionize the way we approach environmental issues. The ability of quadrupole time-of-flight MS instruments to acquire full mass range spectra without sacrificing speed or sensitivity makes these types of instruments an excellent choice for qualitative and quantitative analyses across a wide range of antimicrobial classes in the presence of complex matrices. However, while the high resolving power of quadrupole time-of-flight MS provides a high degree of selectivity through exact mass measurements, this MS format has generally lower sensitivity compared to triple quadrupole MS when running under selected reaction monitoring mode. On the other hand, the Orbitrap™ MS overcomes many limitations that other LC-MS instruments have because it can use the synchronous full-scan MS and MS/MS acquiring capabilities, which are advantageous on both confirmation and quantification. While the quadrupole time-of-flight MS can also perform full-scan MS and MS/MS experiments, the Orbitrap™ MS has a much faster data acquisition rate that can provide low detection limits and higher sensitivities, allowing detection of low signal intensity ions on antimicrobials and their transformation products. Orbitrap™ MS cost about twice as much as the other MS platforms, making this a rare instrument in many environmental laboratories. Therefore, high-resolution MS technologies are still considered research tools with very limited applications in environmental regulatory settings.

Need for Complementary Bioanalytical and Molecular Assays to Assess Impacts of Manufacturing Wastes

Environmental issues require a comprehensive environmental evaluation through combined bioanalytical approaches with exposure and hazard analysis. In the context of AMR, this would require combining MS (targeted vs screening/retrospective) focused on chemical targets with bioanalytical approaches focused on the selective effect, i.e. measuring phenotypic resistance or the increase in resistance genes. In addition, ecotoxicity tests should be implemented as part of the standard test, using whole organisms (fish assays), bacteria, or cell toxicity assays.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Monitoring antimicrobial resistance genes in environmental matrices was recently recommended because there is increasing recognition that these genes can represent emerging contaminants.[ADDIN EN.CITE
<EndNote><Cite><Author>Pruden</Author><Year>2006</Year><RecNum>114</RecNum><DisplayText><style face="superscript">[197]</style></DisplayText><record><rec-number>114</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523972311">114</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pruden, A.</author><author>Pei, R.</author><author>Storteboom, H.</author><author>Carlson, K. H.</author></authors></contributors><auth-address>Department of Civil and Environmental Engineering, Colorado State University, Fort Collins, Colorado 80523, USA. apruden@engr.colostate.edu</auth-address><titles><title>Antibiotic resistance genes as emerging contaminants: studies in northern Colorado</title><secondary-title>Environ Sci Technol</secondary-title></titles><periodical><full-title>Environ Sci Technol</full-title></periodical><pages>7445-50</pages><volume>40</volume><number>23</number><edition>2006/12/22</edition><keywords><

keyword>Colorado</keyword><keyword>Drug Resistance, Microbial/*genetics</keyword><keyword>*Environmental Microbiology</keyword><keyword>Environmental Monitoring/*statistics & numerical data</keyword><keyword>Fresh Water/*microbiology</keyword><keyword>Genes, Bacterial/*genetics</keyword><keyword>Geologic Sediments/*microbiology</keyword><keyword>Polymerase Chain Reaction</keyword><keyword>RNA, Ribosomal, 16S/genetics</keyword><keyword>Seasons</keyword><keyword>Sequence Analysis, DNA</keyword><keyword>Water Pollutants, Chemical/*analysis</keyword><keyword>*Water Supply</keyword></keywords><dates><year>2006</year><pub-dates><date>Dec 1</date></pub-dates></dates><isbn>0013-936X (Print)
0013-936X (Linking)</isbn><accession-num>17181002</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/17181002</url></related-urls></urls></record></Cite></EndNote>] Molecular analyses of environmental samples to identify the presence and diversity of resistance genes could potentially become very useful in identifying hotspots of AMR locally and on a global scale.[ADDIN EN.CITE <EndNote><Cite><Author>Luby</Author><Year>2016</Year><RecNum>546</RecNum><DisplayText><style face="superscript">[100]</style></DisplayText><record><rec-number>546</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529501413">546</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Luby, Elizabeth</author><author>Ibekwe, A. Mark</author><author>Zilles, Julie</author><author>Pruden, Amy</author></authors></contributors><titles><title>Molecular Methods for Assessment of Antibiotic Resistance in Agricultural Ecosystems: Prospects and Challenges</title><secondary-title>Journal of Environmental Quality</secondary-title></titles><periodical><full-title>Journal of Environmental Quality</full-title></periodical><pages>441-453</pages><volume>45</volume><number>2</number><dates><year>2016</year></dates><urls><related-urls><url>http://dx.doi.org/10.2134/jeq2015.07.0367</url></related-urls></urls><electronic-resource-num>10.2134/jeq2015.07.0367</electronic-resource-num><language>English</language></record></Cite></EndNote>] Genetic data, particularly based on culture-independent approaches, holds particular promise for environmental AMR studies because of its ability to more broadly capture the signature of environmental samples.[ADDIN EN.CITE <EndNote><Cite><Author>Bengtsson-

Palme</Author><Year>2017</Year><RecNum>561</RecNum><DisplayText><style face="superscript">[198]</style></DisplayText><record><rec-number>561</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1529503004">561</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bengtsson-Palme, Johan</author><author>Larsson, D. G. Joakim</author><author>Kristiansson, Erik</author></authors></contributors><titles><title>Using metagenomics to investigate human and environmental resistomes</title><secondary-title>Journal of Antimicrobial Chemotherapy</secondary-title></titles><periodical><full-title>Journal of Antimicrobial Chemotherapy</full-title></periodical><pages>2690-2703</pages><volume>72</volume><number>10</number><dates><year>2017</year></dates><isbn>0305-7453</isbn><urls><related-urls><url>http://dx.doi.org/10.1093/jac/dkx199</url></related-urls></urls><electronic-resource-num>10.1093/jac/dkx199</electronic-resource-num></record></Cite></EndNote>] Genomic research tools are more accessible to researchers in developed countries, but the falling cost of next generation sequencing is increasing the access to and use of such approaches to unravel the complexities of antimicrobial resistance.

AMR is a global challenge, so establishing global monitoring networks for AMR determinants would help to understand the dynamics of AMR in the environmental context. Global data collection should be open and include shared ways to sample, prepare, analyze and interpret samples. First, key AMR determinants need to be evaluated comprehensively, and AMR markers selected for local and global monitoring.

F. What information is needed to establish a standard for acceptable waste discharge from a manufacturing facility?

Implementing acceptable waste discharge standards requires:

- Defining a standard (i.e., a maximum discharge limit)
- Identifying the manufacturing practices (or mitigation strategies) required to meet the standard
- Assessing and evaluating manufacturing practices by monitoring discharge

Defining Discharge Limits

There are no regulatory standards for antibiotic waste discharges. Ultimately, it boils down to what standard is a safe standard; however, the term "safe" can also be open to interpretation. To determine "safe" or "acceptable," we must decide if the goal is to protect human health, environmental health, or both. The approach applied to reach safe standards may be different based on the goal.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

A lofty goal is to adopt a zero discharge standard, which would be considered safe. However, this standard might not realistically apply everywhere, especially with antimicrobial production that generates very large liquid waste volumes. The answer is likely in the middle—an acceptable standard that allows discharges of waste, but at a safe limit to protect human and environmental health. Table 3 proposes several assays with corresponding metrics as methods to identify safe limits.

These safety limit proposals establish environmental concentrations that can be measured in the environmental waters near manufacturing sites or in the effluent itself. Each of the assays use a different methodology, but there is some agreement between the assays published for ciprofloxacin and tetracycline.[ADDIN EN.CITE ADDIN EN.CITE.DATA] It is important to note that the safety limits set in the assays described by Gullberg, Lundstrom, and Kraupner must be established for each antimicrobial. In addition, when concentration limits are used, more information is needed about where the sample is collected (e.g., effluent at the point of discharge, or further downstream).

A review and meta-analysis of risk assessment studies by Le Page et al[ADDIN EN.CITE ADDIN EN.CITE.DATA] concluded that environmental risk assessment (based on one cyanobacteria species) is insufficient and further data on the effects of antimicrobials on bacterial diversity, community structure, and ecosystem function are needed. Based on the few data available, the authors reported a conservative limit of 154 ng/L based on data from 27 antimicrobials and no observed effect concentration data for a range of sensitive phyla. For implementation, the authors suggest an antimicrobial discharge threshold limit of 100 ng/L would be protective of environmental bacterial populations.

Some manufacturers propose mass balance-based calculations to estimate the release of antimicrobials during production. In this case, antimicrobial loss or discharge are reported as a percent of the total drug produced. Concerns with this approach are that the measurement does not reflect the concentration of discharged drug in environmental waters and the failure to apply functional limits could result in concentrations of discharge that will select for resistance in the environment microbial environment. However, mass balance calculations may have a value in detecting comparably large losses of active compounds during the manufacturing.

Effluents from manufacturing plants can be harmful when disposed in ways that apply selective pressure on natural microbial communities. In many cases, third party wastewater treatment companies manage producers' effluents, which are then mixed with human waste. The human health or environmental risk of this discharge flow is not well understood. Manufacturers also provide grey water, mycelial mats, and other biosolids containing antimicrobials or active antimicrobial metabolites to the local agricultural economy. Restrictions or measures applied to these activities should be considered when developing an intervention strategy. This information gap relating to community practices and economic impact must be addressed when considering intervention requirements.

In addition to the efforts described here, the AMR Industry Alliance [ADDIN EN.CITE <EndNote><Cite><Year>2017</Year><RecNum>463</RecNum><DisplayText><style face="superscript">[203]</style></DisplayText><record><rec-number>463</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524664909">463</key></foreign-keys><ref-type name="Web Page">12</ref-type><contributors><authors><author>AMR Industry Alliance,</author></authors></contributors><titles><title>AMR Industry Alliance</title></titles><volume>2018</volume><dates><year>2017</year></dates><publisher>AMR Industry Alliance</publisher><urls><related-urls><url>https://www.amrindustryalliance.org/</url></related-urls></urls></record></Cite></EndNote>] is working towards developing discharge limit target values, in collaboration with WHO. Currently, the WHO is organizing a scientific expert meeting to discuss available data and additional data needed to set standardized targets for waste discharge. India´s government is also planning to set national discharge limits, as indicated in their National Action Plan for AMR.[ADDIN EN.CITE <EndNote><Cite><Year>2017</Year><RecNum>520</RecNum><DisplayText><style face="superscript">[204]</style></DisplayText><record><rec-number>520</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527861800">520</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><secondary-authors><author>Ministry of Health & Family Wellfare, Government of India</author></secondary-authors></contributors><titles><title>National Action Plan on Antimicrobial Resistance</title></titles><dates><year>2017</year></dates><urls><related-

urls><url>http://www.searo.who.int/india/topics/antimicrobial_resistance/nap_amr.pdf</url></related-urls></urls></record></Cite></EndNote>]

Required Industrial Interventions to Meet a Standard

When a discharge standard and evaluation measures are defined and implemented, a critical next step for industry is to identify the most cost-efficient interventions and pinpoint when and where to intervene in the production process in order to meet that standard (e.g., avoiding contamination of waste-streams, pre-treatment of certain waste-streams, or treatment at the point of discharge). The variability of operational practices and waste management protocols among manufacturing facilities will likely lead to different measures to meet the standards.

One challenge for cost-effective implementation is that many companies are reluctant to share information on how they treat or manage effluent and biosolid manufacture waste. For instance, out of 18 companies assessed by the AMR Benchmark in the area of Manufacturing & Production, 15 have put in place an environmental risk-management strategy. Of these, 12 disclose their strategies publicly. According to the AMR Benchmark, “making such disclosures is an important first step. It provides a measure of transparency, showing the willingness of pharmaceutical companies to adjust their manufacturing practices in order to minimize antibiotic resistance.” Beyond disclosing the strategies, no company disclosed: (1) results of audits on this strategy of the company’s manufacturing sites; 2) results of audits on this strategy of third parties’ manufacturing sites of antibiotic API and drug products and of wastewater treatment plants; 3) the identities of its third parties manufacturing antibiotic API and drug products, and antibiotic waste treatment plants; 4) the levels of antibiotic discharge. Shionogi committed to disclosing its third parties in its 2017 environment, health, and safety report. Greater transparency of this information from companies would help to rapidly and more cost-efficiently intervene in the production process. It would also help determine the most appropriate intervention strategies.

The financial impact to the facility also factors into identifying intervention requirements. It is likely that even small mitigation strategies could have a high impact, without the need to implement higher-cost interventions such as ultraviolet radiation or reverse-osmosis treatment of effluent waste.

Assessment and Evaluation of Mitigation Practices

If standards are adopted and manufacturing facilities implement interventions to meet these standards, then transparent data on antimicrobial discharge are needed to know when a sufficient and justified level of protection is achieved. Once emissions are reduced, it is unclear how long it will take an area to recover (i.e., revert to a base-line concentration of drug) after ongoing discharge of antimicrobials in the environment. This may affect our ability to measure progress and impact accurately. To evaluate long-term environmental recovery, metrics and timeframe estimates are needed in order to inform the current selection real-time assessment practices and determine if mitigation should be managed based on risk or hazard. A critically important piece is that discharges that have contributed to the expansion of resistance or the evolution of novel resistance, are likely not reversible, similar to resistance found in hospitals and on the farm.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Once a new form of resistance develops in a pathogen it will likely remains within the environmental resistome where it may amplify and spread, potentially affecting human health.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Incentives and Regulation for Mitigation Practices

Incentives and regulations would help to promote good manufacturing practice that minimizes the impact of antibiotic manufacturing discharge on the environment. The AMR Benchmark, [ADDIN EN.CITE <EndNote><Cite><Year>2018</Year><RecNum>464</RecNum><DisplayText><style face="superscript">[209]</style></DisplayText><record><rec-number>464</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524665340">464</key></foreign-keys><ref-type name="Web Page">12</ref-type><contributors><authors><author>AMR Benchmark,</author></authors></contributors><titles><title>AMR Benchmark</title></titles><volume>2018</volume><dates><year>2018</year></dates><publisher>Access to Medicine Foundation</publisher><urls><related-urls><url>https://amrbenchmark.org/</url></related-urls></urls></record></Cite></EndNote>] which incentivizes disclosure of waste management and discharge data, can provide the basis for green procurement of antibiotics, the preferential purchase and use of antibiotics produced in facilities that adopt best practices for reducing emissions. Regulatory practices and capacity vary worldwide, and, unfortunately, are most lacking in areas where these policies would be most beneficial. An exception is the intention of the Indian government to set and implement such standards by 2020. However, governments, policy organizations, the scientific community, and the pharmaceutical industry will need to work together to identify best practices, which include:

- Ssetting standards
- Communicating appropriate measures for that standard

- Informing facilities how best to develop procedural changes or apply interventions within their manufacturing process to meet those standards
- Identifying evaluation standards and who performs assessments
- Developing accountability guidelines for practicing these strategies within their facilities and supply chains
- Providing a system for collective reporting of data and progress

Antimicrobials Used as Crop Pesticides

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Summary

- Antimicrobials are commonly applied across the globe as pesticides to manage crop disease. Some medically important antimicrobials are used on crops to prevent or treat plant diseases.
- Information around the type and amount of antimicrobials used on crops varies by country and is not captured globally. More research is needed to determine whether using antibiotics as pesticides selects for resistance in bacteria that affects human health. Few studies have been done.
- Studies suggest that use of triazole fungicides can lead to resistance in the environmental fungus *Aspergillus fumigatus*, which can cause human infections resistant to antifungal medications.
- Using antimicrobials as pesticides could contribute to resistant microorganisms in the environment, which could be concerning if the microorganism can cause an infection in people, or if antimicrobial resistance (AMR) develops and shares resistance to antimicrobials commonly used to treat human infections.
- Antimicrobial agents, like copper, are not used in human medicine but may contribute to resistance to antimicrobials used in human medicine.
- There are strategies to avoid or limit the use of medically important antimicrobials as pesticides, including modeling to predict high-risk periods for crop disease, control practices that prevent the spread of crop pathogens, and alternative treatments. However, these strategies are not always used globally and growers need support to use them, such as access to these treatments and training.

Filling Knowledge Gaps

Scientific review suggests that the following actions could improve understanding and guide action:

- Conduct research to determine the potential impact of antimicrobial pesticide exposure on the human, plant, and animal microbiomes after pesticide application
- ~~Conduct research to determine the impact of antimicrobial pesticide exposure on the human, plant, and animal microbiomes~~
- Identify and promote best management practices for applying antimicrobials as pesticides to minimize exposure to humans, animals, and the surrounding environment.
- Establish greater global transparency of antimicrobial use as pesticides by collecting information like the amount of antimicrobial used on crops each year.

- S~~Through international collaborations, s~~hare data between countries on the relative efficacy of antimicrobials as pesticides and potential alternatives, so that antimicrobials used in human medicine are only considered when there is evidence of efficacy and no alternatives are available.
- Conduct studies to develop efficacious and feasible alternatives to antimicrobials to prevent or treat crop disease and identify strategies to ensure that alternative treatments are available to growers.
- Identify and develop appropriate and reproducible methods to monitor the crop field and surrounding environment to determine if there are increases in antimicrobial resistance when medically important antimicrobials are used and when co-selection is a concern.
- Consider~~ation should be given to~~ updating national AMR action plans to include antimicrobial stewardship principles for using antimicrobials as pesticides with actions that are based upon country-specific practices.

Background Statement

Antimicrobials are widely used as pesticides for crop disease management. In some cases, these antimicrobials are the same, or closely related to, antimicrobials used in human medicine (e.g., tetracyclines, aminoglycosides, and triazoles). Using antimicrobials as crop pesticides has the potential to select for resistant microbes present in the environment. This is of particular concern if the microbe can cause human infection or confers transferable resistance mechanisms to antimicrobials commonly used to treat human infections. For example, using streptomycin as a pesticide could select for transmissible streptomycin resistance in environmental bacteria, such as an aminoglycoside phosphatase encoded by the tandem gene pair *strA-strB* carried on plasmids.[ADDIN EN.CITE <EndNote><Cite><Author>Snelders</Author><Year>2012</Year><RecNum>469</RecNum><DisplayText><style face="superscript">[210, 211]</style></DisplayText><record><rec-number>469</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527126326">469</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Snelders, Eveline</author><author>Camps, Simone M. T.</author><author>Karawajczyk, Anna</author><author>Schaftenaar, Gijs</author><author>Kema, Gert H. J.</author><author>van der Lee, Henrich A.</author><author>Klaassen, Corné H.</author><author>Melchers, Willem J. G.</author><author>Verweij, Paul E.</author></authors></contributors><titles><title>Triazole Fungicides Can Induce Cross-Resistance to Medical Triazoles in Aspergillus fumigatus</title><secondary-title>PLOS ONE</secondary-title></titles><periodical><full-title>PLoS One</full-title></periodical><pages>e31801</pages><volume>7</volume><number>3</number><dates><year>2012</year></dates><publisher>Public Library of Science</publisher><urls><related-

urls><url>https://doi.org/10.1371/journal.pone.0031801</url></related-urls></urls><electronic-resource-num>10.1371/journal.pone.0031801</electronic-resource-num></record></Cite><Cite><Author>Jones</Author><Year>2000</Year><RecNum>443</RecNum><record><rec-number>443</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145418">443</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Jones, A. L., and E. L. Schnabel</author></authors><tertiary-authors><author>J. L. Vanneste </author></tertiary-authors></contributors><titles><title>The development of streptomycin-resistant strains of Erwinia amylovora</title><secondary-title>Fire Blight: The disease and its causative agent, Erwinia amylovora</secondary-title></titles><section>235-251</section><dates><year>2000</year></dates><publisher>CAB International</publisher><urls></urls></record></Cite></EndNote>] New types of plasmid-mediated resistance, which confer~~s~~ resistance to all of the aminoglycosides, have been emerging in bacteria causing healthcare-associated infections. This type of resistance, a 16S-methylases gene, has not been found in plant agriculture, but vigilance is needed to ensure use of medically important antibiotics on crops does not ultimately affect the ability to treat serious infections in people. Of particular concern are cases where antibiotic use on crops increases or when the environment exposed to the pesticide is contaminated with multi-drug resistant microbes.

Aspergillus fumigatus is a fungus common in the environment. In the last decade, infections with *Aspergillus fumigatus*~~, with~~ resistan~~ce~~ to all triazole antifungals, were detected first in Europe and now across the world. This fungus infects humans through inhalation, causing severe and often fatal invasive mold infections in the growing proportion of the world's population that is immunocompromised. Triazole fungicides are used widely in plant agriculture, representing the largest class of fungicides in some countries ([HYPERLINK "https://water.usgs.gov/nawqa/pnsp/usage/maps/"] and [HYPERLINK "http://www.fao.org/faostat/en/" \l "data/RP"]). In human medicine, there are triazole antifungal medications that are structurally related to triazole crop fungicides. These medications are used to treat superficial skin infections and many life-threatening fungal diseases. Triazole antifungals have become the mainstay of therapy for these infections; however, these medications are ineffective against resistant strains, associated with higher mortality.[ADDIN EN.CITE <EndNote><Cite><Author>Verweij</Author><Year>2016</Year><RecNum>471</RecNum><DisplayText><style face="superscript">[212]</style></DisplayText><record><rec-number>471</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"

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timestamp="1527126481">471</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Verweij, Paul E.</author><author>Chowdhary, Anuradha</author><author>Melchers, Willem J. G.</author><author>Meis, Jacques F.</author></authors></contributors><titles><title>Azole Resistance in Aspergillus fumigatus: Can We Retain the Clinical Use of Mold-Active Antifungal Azoles?</title><secondary-title>Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America</secondary-title></titles><periodical><full-title>Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America</full-title></periodical><pages>362-368</pages><volume>62</volume><number>3</number><dates><year>2016</year><pub-dates><date>10/20
09/01/received
10/02/accepted</date></pub-dates></dates><publisher>Oxford University Press</publisher><isbn>1058-4838
1537-6591</isbn><accession-num>PMC4706635</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4706635/</url></related-urls></urls><electronic-resource-num>10.1093/cid/civ885</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] There are several lines of evidence that suggest agricultural and other environmental triazole use has selected for the most common type of pan-triazole-resistant *A. fumigatus* infections, known as TR34/L98H ([HYPERLINK "http://ecdc.europa.eu/en/publications-data/risk-assessment-impact-environmental-usage-triazoles-development-and-spread"]). [ADDIN EN.CITE ADDIN EN.CITE.DATA] Notably, the majority of patients with resistant infections did not have previous exposure to medical triazole antifungals,[ADDIN EN.CITE <EndNote><Cite><Author>van der Linden</Author><Year>2011</Year><RecNum>470</RecNum><DisplayText><style face="superscript">[214]</style></DisplayText><record><rec-number>470</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527126405">470</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>van der Linden, Jan W. M.</author><author>Snelders, Eveline</author><author>Kampinga, Greetje A.</author><author>Rijnders, Bart J. A.</author><author>Mattsson, Eva</author><author>Debets-Ossenkopp, Yvette J.</author><author>Kuijper,</author><author>Van Tiel, Frank H.</author><author>Melchers, Willem J. G.</author><author>Verweij, Paul E.</author></authors></contributors><titles><title>Clinical Implications of Azole Resistance in Aspergillus fumigatus, the Netherlands, 2007–2009</title><secondary-title>Emerging Infectious Diseases</secondary-title></titles><periodical><full-

title>Emerging Infectious Diseases</full-title></periodical><pages>1846-1854</pages><volume>17</volume><number>10</number><dates><year>2011</year></dates><publisher>Centers for Disease Control and Prevention</publisher><isbn>1080-6040
1080-6059</isbn><accession-num>PMC3311118</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3311118/</url></related-urls></urls><electronic-resource-num>10.3201/eid1710.110226</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>] suggesting that they became infected with a strain already carrying the mutation. The source of resistant pathogen is unknown, but it is unlikely that the patients were infected with a susceptible strain that developed resistance in vivo.

When evaluating the risk of antimicrobial use as a pesticide on human health, it is important to assess:

- The likelihood an antimicrobial selects for resistance to the drug itself
- Resistance to related drugs (i.e., cross-resistance)
- Resistance to unrelated drugs because of genetic linkages between resistance determinants (i.e., co-selection of resistance)
- Potential for transmission of the antimicrobial resistance to human pathogens

It is also important to understand the following:

- The extent to which antimicrobials used as pesticides can contaminate the environment beyond field borders
- How long the antimicrobial is active in the environment
- If antimicrobials within a crop field pose a risk to personnel working in or nearby the field (e.g., adverse health events from microbiome disruption)

Efforts to mitigate the risk of using antimicrobials as pesticides will require the following information:

- The extent to which drugs are used
- Application strategies with proven effectiveness in limiting human exposure
- Strategies that can be used to reduce or eliminate the need to use antimicrobials on crops

Scientific Issues

A. What is the current landscape of antimicrobial use as pesticides; which drugs and how much?

This section describes antimicrobials applied to agricultural crops for management of plant diseases that are the same or closely related to antimicrobials used to treat human infections (Table 4). Some of these antimicrobials are also used in animal agriculture and aquaculture. Antimicrobials used on plants that are not used clinically or on animals will not be addressed, with the exception of copper. Copper formulations are the most commonly used pesticide to prevent bacterial and fungal plant diseases. While copper formulations are not used in human medicine, they may be involved in co-selection of antimicrobial resistance determinants.

Why Antimicrobials are used on Crop Plants

Antibiotics

Bacterial diseases in crop plants can be difficult to control and extremely damaging, impacting the income of farms if left untreated. Following the discovery of antibiotics, several compounds were evaluated for their ability to control bacterial diseases in plants (e.g. penicillin, streptomycin, aureomycin, chloramphenicol, and oxytetracycline). [ADDIN EN.CITE <EndNote><Cite><Author>McManus</Author><Year>2002</Year><RecNum>466</RecNum><DisplayText><style face="superscript">[215]</style></DisplayText><record><rec-number>466</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527124801">466</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Patricia S. McManus</author><author>Virginia O. Stockwell</author><author>George W. Sundin</author><author>Alan L. Jones</author></authors></contributors><titles><title>ANTIBIOTIC USE IN PLANT AGRICULTURE</title><secondary-title>Annual Review of Phytopathology</secondary-title></titles><periodical><full-title>Annual Review of Phytopathology</full-title></periodical><pages>443-465</pages><volume>40</volume><number>1</number><keywords><keyword>antibiotic resistance,Erwinia amylovora,Pseudomonas,Xanthomonas,streptomycin,tetracycline,Tn5393</keyword></keywords><dates><year>2002</year></dates><accession-num>12147767</accession-num><urls><related-urls><url>https://www.annualreviews.org/doi/abs/10.1146/annurev.phyto.40.120301.093927</url></related-urls></urls><electronic-resource-num>10.1146/annurev.phyto.40.120301.093927</electronic-resource-num></record></Cite></EndNote>] Of the antibiotics tested, streptomycin provided excellent control of several bacterial diseases when applied at low doses (100 ppm), was non-toxic to plants, and did not cause undesirable markings on fruit. It was the first antibiotic registered in the U.S. for plant protection in 1958.

Generally, antibiotics are used to control bacterial diseases in high-value crops, primarily tree fruits. Most bacterial plant pathogens are systemic, seed- or tuber-transmitted, while others are present in the environment and overwinter in infected tissues. A bacterial plant pathogen needs a fresh surface wound or natural opening to infect a plant, such as stomata or secretion pores. The wound or opening allows the bacterium to access internal plant tissues. Activities that could cause a wound include weather events (e.g., freeze damage, hailstorms, and wind), insect activity, or horticultural practices, such as pruning trees or damage from machinery.

For many bacterial plant diseases, another important step in the infection process is the epiphytic growth phase, where the pathogen grows on the surface of the plant and multiplies into large population sizes prior to tissue infection (~1,000,000 colony forming units). Environmental conditions influence the growth rate of the pathogen. Unfavorable conditions can reduce pathogen growth, making infection unsuccessful. During the pre-infection epiphytic growth phase, the pathogens are exposed on plant surfaces and vulnerable to disease control methods. Antibiotics are generally applied as a prophylactic (preventive) treatment. The antibiotics disrupt the epiphytic growth phase and prevent subsequent infection.

Using antibiotics is discouraged once disease symptoms are visible because antibiotics do not cure the plant when sprayed on infected plants. Additionally, the potential for selection of antibiotic-resistant plant pathogens increases as the population size of the pathogen in host tissues increases.

Antifungals

Fungi makes up the largest group of plant pathogens. Fungicides are used widely in plant agriculture to prevent and treat fungal diseases. Triazoles are widely used as fungicides ([HYPERLINK "http://www.apsnet.org/publications/apsnetfeatures/Pages/Fungicides.aspx"]). The triazoles have broad-spectrum antifungal activity, are systemic (absorbed, redistributed, and active within leaves), and require fewer applications than contact fungicides for disease control. Triazoles are used on a diverse range of plants. Previously they were largely used in high-value crops, such as orchard trees and grapes, but now they are increasingly used on commodity crops like wheat, corn, and soybeans. For example, in 1995, the low estimate of triazole use in the U.S. on orchards and grapes (134 metric tons) was more than double the use on wheat and corn (use on soybeans was not reported). In 2015, the preliminary low estimates for use on wheat (1,068 metric tons), corn (331), and soybeans (206) exceeded the use on orchards and grapes (202). ~~When triazole use estimates across all crops are considered over time, low and high estimates from 2006 to 2015 appear to have increased approximately five-fold (~~[HYPERLINK "https://water.usgs.gov/nawqa/pnsp/usage/maps/"]). The increase ~~may have~~ occurred in part because

Commented [SM3]: I am still concernd about the relevancy of a 10 year old citation here. USDA NASS Chemical Use Survey data would be better to reference.

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Commented [SM5]: How were these values obtained from this reference? What AIs were included?

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USGS estimated low and high usage estimates may be downloaded here. The following example links to downloadable maps and data for Tebuconazole usage in 1995, and the user may select low or high estimates. [HYPERLINK "https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=1995&map=TEBUCONAZOLE&hilo=L"]
We downloaded low and high estimates for all available crops from 1995-2015 for the following list of triazole fungicide AIs. Not all AIs are reported in each year for each crop, as the USGS NAWQA data are based on proprietary usage surveys which form the basis of USDA NASS estimates as well. https://pubs.usgs.gov/sir/2013/5009/

Cyproconazole

growers were faced with new and emerging disease such as soybean rust, wheat scab, and corn southern rust.

Publically Available Sources of Pesticide Use Data in the U.S.

Several U.S. government agencies collect or report data on materials applied to plants or agricultural soils: the U.S. EPA ([HYPERLINK "https://www.epa.gov/sites/production/files/2017-01/documents/pesticides-industry-sales-usage-2016_0.pdf"]); the National Agricultural Statistics Service (NASS) within USDA; and the state of California ([HYPERLINK "https://www.cdpr.ca.gov/docs/pur/purmain.htm"]). The National Water-Quality Assessment Project within USGS uses public and proprietary data sources to estimate pesticide use.

The data from NASS Agricultural Chemical Use Program provides information on on-farm chemical use and pest management practices. The chemical use data are collected directly from farmers and includes information like the amount an active ingredient of a pesticide that is used ~~each year~~in the survey year, the number of applications of a material, and the percentage of acreage treated. Data for materials applied to crops is available in the on-line QuickStats database at [HYPERLINK "https://quickstats.nass.usda.gov/"].

The National Water-Quality Assessment Project estimating annual use of pesticides for agriculture based on confidential reports and harvested crop acreage surveys of specific farms located within USDA Crop Reporting Districts. The proprietary farm-specific data are used to project pesticide use in larger regions based on acreage of crops in a region, and reported by the USDA Census of Agriculture.[ADDIN EN.CITE <EndNote><Cite><Author>Baker</Author><Year>2015</Year><RecNum>476</RecNum><DisplayText><style face="superscript">[216]</style></DisplayText><record><rec-number>476</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527168536">476</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Baker, N.T., and Stone, W.W.</author></authors></contributors><titles><title>Estimated annual agricultural pesticide use for counties of the conterminous United States, 2008–12</title><secondary-title>U.S. Geological Survey Data Series 907</secondary-title></titles><dates><year>2015</year></dates><urls></urls><electronic-resource-num>10.3133/ds907</electronic-resource-num></record></Cite></EndNote>] USGS provides annual high and low estimates of pesticide use at [HYPERLINK "https://water.usgs.gov/nawqa/pnsp/usage/maps/county-level/"].

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Soybean rust emerged in the US in 2004 [HYPERLINK "https://www.apsnet.org/edcenter/intropp/lessons/fungi/Basidiomycetes/Pages/SoybeanRust.aspx"]
Wheat scab re-emerged in the mid 1990s [HYPERLINK "https://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS.1997.81.12.1340"]

Commented [SM9]: Has that been demonstrated to cause resistance of phytopathogens or human pathogens? The implication is that this level of usage or usage on these crops specifically is a problem or a potential problem.

Commented [PJ(10R9]: See section labeled, The Threat of Resistance to Human Health on page 75. Also pasted below: When evaluating the risk of using antimicrobials as pesticides and the potential to select for resistant microbes, antibiotics and antifungals should be considered separately because they are chemically distinct and target different types of microbes. For antibiotics, the very limited and special uses, apple and pear application, application to low density microbial habitats, and the low bioavailability would argue against the likelihood for significant resistance selection. The risk to human health from antibiotics applied on plants should be very low, and certainly so compared to the many other (non-crop) environmental sources for antibiotic resistance selection. Triazoles have a much larger, longer, and more diverse use and their stability in the environment would argue for much greater chances for resistance selection, which evidence supports. At present, the concern for antifungal resistance from agricultural fungicide use is largely restricted to *A. fumigatus*, but much remains unknown about other fungal clinical pathogens. For example, an important fungal disease caused by the yeast *Candida auris* has rapidly emerged in several world regions in the last few years. Most isolates of *C. auris* from ill people are resistant to the triazole fluconazole. More research is needed to understand the contribution of use of agricultural triazole fungicides to resistance in medically important fungi and yeasts.

Other data sources with pesticide use outside of the U.S. need to be investigated. Additionally, centralization of data and standards for reporting data are needed to assess the extent to which antimicrobials are used and inform assessments of the possible risk to human health.

Types of Antimicrobials Used in Crop Plants

Antibiotics

Streptomycin. Streptomycin is an aminoglycoside used in human medicine and related to other aminoglycosides used for treatment of serious bacterial infections. Resistance mechanisms that confer resistance to all aminoglycosides have emerged on mobile genetic elements resulting in an increased risk for horizontal gene transfer.

The U.S. has used streptomycin to manage bacterial diseases in plants since the 1950s. Streptomycin may be applied to potato seed pieces or tomato and tobacco transplants in greenhouses, prior to planting in the field, for the prevention of rots. The application of streptomycin on these crops is limited or not allowed after planting outdoors. Table 5 summarizes the registered uses of streptomycin on crops in the U.S.

More than 90% of streptomycin used for crop protection in the U.S. is applied to pear and apple orchards to prevent fire blight caused by *Erwinia amylovora*.[ADDIN EN.CITE <EndNote><Cite><Author>Stockwell</Author><Year>2012</Year><RecNum>448</RecNum><DisplayText><style face="superscript">[217]</style></DisplayText><record><rec-number>448</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145639">448</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Stockwell, V. O.</author><author>Duffy, B.</author></authors></contributors><auth-address>Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97330, USA.</auth-address><titles><title>Use of antibiotics in plant agriculture</title><secondary-title>Rev Sci Tech</secondary-title><alt-title>Revue scientifique et technique (International Office of Epizootics)</alt-title></titles><periodical><full-title>Rev Sci Tech</full-title><abbr-1>Revue scientifique et technique (International Office of Epizootics)</abbr-1></periodical><alt-periodical><full-title>Rev Sci Tech</full-title><abbr-1>Revue scientifique et technique (International Office of Epizootics)</abbr-1></alt-periodical><pages>199-210</pages><volume>31</volume><number>1</number><edition>2012/08/02</edition><keywords><keyword>Anti-Bacterial Agents/*pharmacology</keyword><keyword>Bacteria/*drug effects</keyword><keyword>Crops, Agricultural/*microbiology</keyword><keyword>Erwinia amylovora/drug effects/pathogenicity</keyword><keyword>Plant Diseases/microbiology/*prevention & control</keyword></keywords><dates><year>2012</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>0253-1933 (Print)
0253-1933</isbn><accession-num>22849276</accession-num><urls></urls><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] Streptomycin is also registered for fire blight management in Canada, Israel, Mexico, and New Zealand. It was used in Austria, Germany, and Switzerland on a strictly-regulated, emergency use basis to control and prevent fire blight until 2016, after which the material is no longer approved in Switzerland and the EU. [ADDIN EN.CITE ADDIN EN.CITE.DATA]

Fire blight is the most destructive bacterial disease of pear and apple. Trees are most vulnerable to infection by *E. amylovora* during bloom in the spring months. The bacterial pathogen survives the winter months in cankers (infections on the trunk and stems of trees). In the spring, pathogen cells ooze from cankers and insects, wind, and rain spread them to open flowers. The pathogen colonizes the nutrient-rich stigmas and rapidly develops population sizes exceeding 10^6 colony-forming units per flower under favorable weather conditions.[ADDIN EN.CITE <EndNote><Cite><Author>Thomson</Author><Year>2000</Year><RecNum>472</RecNum><DisplayText><style face="superscript">[219]</style></DisplayText><record><rec-number>472</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527127177">472</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>S. V. Thomson</author></authors><secondary-authors><author>J. L. Vanneste </author></secondary-authors></contributors><titles><title>Epidemiology of fire blight</title><secondary-title>Fire blight: the disease and its causative agent, Erwinia amylovora</secondary-title></titles><dates><year>2000</year></dates><pub-location>Wallingford, UK</pub-location><publisher>CAB International</publisher><urls></urls></record></Cite></EndNote>] Moisture (rain or heavy dew) helps the pathogen move to the nectary tissue of the flower, where *E. amylovora* invades the plant tissues through the nectarthodes (nectar secreting pores). Inside the intercellular spaces of the flower, the pathogen produces effector proteins that kill plant tissues, while migrating down the floral stem into the branches. Soon, the disease kills flower clusters and the symptoms of fire blight are visible. At this stage, diseased and surrounding healthy tissues should be removed to reduce internal spread. Secondary phases of the disease include infecting young shoots or fruits. The spread of

fire blight from infected branches from floral or shoot infections to the trunk can be lethal. Young trees in orchards or nurseries are especially vulnerable to fire blight. Regional losses to growers during widespread outbreaks of fire blight are in the estimated range of $40 million to $70 million.[ADDIN EN.CITE ADDIN EN.CITE.DATA] It was estimated that growers across the U.S. spend at least $100 million annually to fight this disease. [ADDIN EN.CITE
<EndNote><Cite><Author>Norelli</Author><Year>2003</Year><RecNum>450</RecNum><DisplayText><style face="superscript">[221]</style></DisplayText><record><rec-number>450</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145756">450</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Norelli, John L.</author><author>Jones, Alan L.</author><author>Aldwinckle, Herb S.</author></authors></contributors><titles><title>Fire Blight Management in the Twenty-first Century: Using New Technologies that Enhance Host Resistance in Apple</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>756-765</pages><volume>87</volume><number>7</number><dates><year>2003</year><pub-dates><date>2003/07/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS.2003.87.7.756</url></related-urls></urls><electronic-resource-num>10.1094/PDIS.2003.87.7.756</electronic-resource-num><access-date>2018/04/19</access-date></record></Cite></EndNote>]

The discovery that streptomycin was effective against fire blight provided growers a method to control the disease; however, the epidemiology of the pathogen and the disease were not well understood in the 1960's. Growers tended to spray streptomycin frequently during the growing season. There were reports of failures to control fire blight using streptomycin within 20 years after streptomycin was first used in pear and apple orchards.[ADDIN EN.CITE
<EndNote><Cite><Author>Schroth</Author><Year>1979</Year><RecNum>479</RecNum><DisplayText><style face="superscript">[222]</style></DisplayText><record><rec-number>479</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527171860">479</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schroth, M. N.</author></authors></contributors><titles><title>Streptomycin resistance in Erwinia amylovora</title><secondary-title>Phytopathology</secondary-title></titles><periodical><full-

title>Phytopathology</full-title></periodical><pages>565-568</pages><volume>69</volume><dates><year>1979</year><pub-dates><date>1979</date></pub-dates></dates><urls><related-urls><url>https://ci.nii.ac.jp/naid/30040910061/en/</url></related-urls></urls><electronic-resource-num>10.1094/Phyto-69-565</electronic-resource-num></record></Cite></EndNote>] Streptomycin resistance in *E. amylovora* has been reported subsequently in many regions of the U.S., Canada, Israel, Mexico, and New Zealand.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Frequently, streptomycin resistance in *E. amylovora* is due to a spontaneous mutation in a gene known as *rpsL,* which leads to a substitution of lysine to arginine at codon 43 [K43R].[ADDIN EN.CITE <EndNote><Cite><Author>Jones</Author><Year>1995</Year><RecNum>473</RecNum><DisplayText><style face="superscript">[224]</style></DisplayText><record><rec-number>473</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527127866">473</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Chien-Shun Chiou & A. L. Jones</author></authors></contributors><titles><title>Molecular Analysis of High-Level Streptomycin Resistance in Erwinia amylovora</title><secondary-title>Molecular Plant Pathology</secondary-title></titles><periodical><full-title>Molecular Plant Pathology</full-title></periodical><pages>324–328</pages><volume>85</volume><number>3</number><dates><year>1995</year></dates><urls></urls></record></Cite></EndNote>] In Michigan, isolates of *E. amylovora* also gained resistance to streptomycin through an acquired tandem gene pair *strA-strB*, which encodes for an aminoglycoside phosphatase that inactivates the antibiotic.[ADDIN EN.CITE <EndNote><Cite><Author>Chiou C-S</Author><Year>1995</Year><RecNum>474</RecNum><DisplayText><style face="superscript">[215, 225]</style></DisplayText><record><rec-number>474</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527127993">474</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Chiou C-S, Jones AL</author></authors></contributors><titles><title>Expression and identification of the strA-strB gene pair from streptomycin-resistant Erwinia amylovora</title><secondary-title>Gene</secondary-title></titles><periodical><full-title>Gene</full-title></periodical><pages>47–51</pages><volume>152</volume><dates><year>1995</year></dates><urls></urls></record></Cite><Cite><Author>McManus</Author><Year>2002</Year><RecNum>466</RecNum><record><rec-number>466</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527124801">466</key></foreign-keys><ref-

type name="Journal Article">17</ref-type><contributors><authors><author>Patricia S. McManus</author><author>Virginia O. Stockwell</author><author>George W. Sundin</author><author>Alan L. Jones</author></authors></contributors><titles><title>ANTIBIOTIC USE IN PLANT AGRICULTURE</title><secondary-title>Annual Review of Phytopathology</secondary-title></titles><periodical><full-title>Annual Review of Phytopathology</full-title></periodical><pages>443-465</pages><volume>40</volume><number>1</number><keywords><keyword>antibiotic resistance,Erwinia amylovora,Pseudomonas,Xanthomonas,streptomycin,tetracycline,Tn5393</keyword></keywords><dates><year>2002</year></dates><accession-num>12147767</accession-num><urls><related-urls><url>https://www.annualreviews.org/doi/abs/10.1146/annurev.phyto.40.120301.093927</url></related-urls></urls><electronic-resource-num>10.1146/annurev.phyto.40.120301.093927</electronic-resource-num></record></Cite></EndNote>]

Despite the potential for resistance to streptomycin, the antibiotic is still used in pear and apple orchards, and remains one of the best chemical controls for fire blight against sensitive isolates of the pathogen. To mitigate resistance, streptomycin is often applied in combination with or rotated with oxytetracycline in U.S. tree fruit orchards. In Latin American countries, streptomycin is sold as a single active ingredient, combined with oxytetracycline, or combined with oxytetracycline and copper (Table 6).

Estimates on the use of streptomycin for crop protection on commercial farms in the U.S. were obtained from the U.S. pesticide use databases cited below. The USGS estimated that between 18,000 to 19,800 kg a.i. (active ingredient) of streptomycin was applied to crops in 2015. Figure 2 provides a summary of streptomycin use from 1991 to 2015 in the U.S. from the NASS QuickStats database. Generally, the quantities and usage patterns of streptomycin were similar over the 24-year period. Table 7 summarizes streptomycin usage in 2015, showing that 92% of the streptomycin used on tree fruits was applied to apple. While the total amounts of streptomycin sprayed on crops provides general information about pesticide use, it is important to consider the average number of applications during a growing season and the percent of the orchard acres that were treated. Table 7 shows that streptomycin was applied twice on average to 26% of the total apple acreage in 2015. Pears were treated an average of three times during the season on 16% of the acreage in 2015 (Table 7). Even though apple trees were sprayed less frequently with streptomycin than pear trees, the much larger acreage of apple orchards (136,358

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HA) accounts for the greater total quantity of streptomycin that was used on apple compared to pear (20,823 HA) (Table 7).

Overall, the total amount of streptomycin applied to U.S. pear and apple orchards is only a fraction of the total amount permitted based on product labels (Table 5). Based on the product labels, growers can apply streptomycin 10 to 15 times during a season on 100% of the acreage. The low use of streptomycin by growers is, in part, due to use of fire blight decision aids and disease risk models such as Maryblyt and Cougarblight.[ADDIN EN.CITE <EndNote><Cite><Author>Jones</Author><Year>1995</Year><RecNum>473</RecNum><DisplayText><style face="superscript">[224, 225]</style></DisplayText><record><rec-number>473</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527127866">473</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Chien-Shun Chiou & A. L. Jones</author></authors></contributors><titles><title>Molecular Analysis of High-Level Streptomycin Resistance in Erwinia amylovora</title><secondary-title>Molecular Plant Pathology</secondary-title></titles><periodical><full-title>Molecular Plant Pathology</full-title></periodical><pages>324–328</pages><volume>85</volume><number>3</number><dates><year>1995</year></dates><urls></urls></record></Cite><Cite><Author>Chiou C-S</Author><Year>1995</Year><RecNum>474</RecNum><record><rec-number>474</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527127993">474</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Chiou C-S, Jones AL</author></authors></contributors><titles><title>Expression and identification of the strA-strB gene pair from streptomycin-resistant Erwinia amylovora</title><secondary-title>Gene</secondary-title></titles><periodical><full-title>Gene</full-title></periodical><pages>47–51</pages><volume>152</volume><dates><year>1995</year></dates><urls></urls></record></Cite></EndNote>] The models estimate disease risk and note when growers should intervene with antibiotic treatment. The models use the following parameters: recent history of fire blight in the orchard or surrounding orchards, the occurrence of conducive environmental conditions for rapid growth of the fire blight pathogen on floral tissues, and presence of open flowers on trees.[ADDIN EN.CITE ADDIN EN.CITE.DATA] The decision aids help growers optimize the timing of streptomycin sprays to periods when they will be most effective. This also reduces excessive use of streptomycin and selection pressure for resistance.

In the U.S., the EPA recently granted emergency use registrations for streptomycin on citrus in the states of Florida and limited, specific regions of California to manage a disease called citrus greening, or huanglongbing. The EPA grants emergency use registrations in response to applications from individual states for specific crops and justified that no alternatives are available and efficacious, in addition to economic loss in yield and revenue for the state. Emergency use registrations are time-limited and the quantities and methods for streptomycin use are regulated, which is specified on special use labels. Data on using streptomycin on citrus under these restricted emergency uses are not publically available at this time.

In addition to formulated streptomycin products used on commercial farms by certified pesticide applicators, agricultural streptomycin is also available for residential use in products marketed for plant disease control in home gardens. The USGS or USDA databases would not capture these minor uses of streptomycin in non-commercial agricultural settings. The amount of streptomycin that homeowners use in home garden settings is not known.

Oxytetracycline. Oxytetracycline is a thermostable member of the tetracycline group of antibiotics. Tetracyclines are commonly used in human medicine (e.g., doxycycline) and resistance to one tetracycline often confers resistance to other tetracyclines.[ADDIN EN.CITE <EndNote><Cite><Author>C.</Author><Year>2005</Year><RecNum>482</RecNum><DisplayText><style face="superscript">[229]</style></DisplayText><record><rec-number>482</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527173355">482</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Roberts, Marilyn C.</author></authors></contributors><titles><title>Update on acquired tetracycline resistance genes</title><secondary-title>FEMS Microbiology Letters</secondary-title></titles><periodical><full-title>FEMS Microbiology Letters</full-title></periodical><pages>195-203</pages><volume>245</volume><number>2</number><dates><year>2005</year></dates><urls><related-urls><url>https://onlinelibrary.wiley.com/doi/abs/10.1016/j.femsle.2005.02.034</url></related-urls></urls><electronic-resource-num>doi:10.1016/j.femsle.2005.02.034</electronic-resource-num></record></Cite></EndNote>]

Oxytetracycline was registered for crop protection in the U.S. in 1972, partially to provide an alternative antibiotic for fire blight management, especially for pear cultivated in regions with streptomycin-resistant populations of *E. amylovora.* Oxytetracycline was also registered to control a damaging disease of peaches and nectarines called bacterial spot, caused by *Xanthomonas campestris* pv. *pruni*. As a crop pesticide, oxytetracycline is formulated as oxytetracycline-HCl or oxytetracycline calcium complex. For fire blight management, growers often combine of oxytetracycline with streptomycin and/or copper and apply the materials as a "tank mix." Although tetracyclines are considered high-risk for resistance development, resistance in fire blight (*E. amylovora*) of apples and pears has not been reported under field conditions, whereas resistance in bacterial leaf spot of peaches and nectarines (*Xanthomonas arabicola* pv *pruni*) has been reported.

To control bacterial spot, oxytetracycline is applied at a dose of 150 ppm on peaches and nectarines. The sprays begin at petal fall and can continue at 4 to 7-day intervals until 21 days before harvest. Depending on the severity of disease, environmental conditions and disease history, up to nine applications of oxytetracycline are permitted each year on peach or nectarine.

To manage fire blight, oxytetracycline is applied at 200 ppm on pear and apple. The applications can begin during early bloom and continue at 3 to 6-day intervals through bloom and weather conditions that favor the disease. Up to six applications of oxytetracycline are permitted on apple, and up to 10 applications are permitted on pear each year. The preharvest interval for oxytetracycline on pear and apple is 60 days.

Figure 3 shows oxytetracycline use in U.S. orchards from 1991 to 2015 with data summarized from the NASS QuickStats database. The use of oxytetracycline was fairly consistent over 20 years, but increased in the last two reporting periods, when the acreage of apple treated increased and a greater number of applications were applied to peach in 2011 (Figure 3). In 2015, the NASS database reported that a total of 12,020 kg of oxytetracycline was applied to orchards (Table 7). The USGS estimated similar quantities, between 12,470 to 13,998 kg oxytetracycline in 2015.

In 2015, oxytetracycline was sprayed most frequently on pear, in part, due to the inherent sensitivity of pear to fire blight and the presence of streptomycin-resistant populations of *E. amylovora* in the western states of the U.S., where the majority of pear is grown commercially (Table 7).[ADDIN EN.CITE <EndNote><Cite><Author>Loper JE</Author><Year>1991</Year><RecNum>475</RecNum><DisplayText><style face="superscript">[220]</style></DisplayText><record><rec-number>475</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"

timestamp="1527166542">475</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Loper JE, Henkels MD, Roberts RG, Grove GG, Willett MJ, Smith TJ</author></authors></contributors><titles><title><style face="normal" font="default" size="100%">Evaluation of streptomycin, oxytetracycline, and copper resistance in </style><style face="italic" font="default" size="100%">Erwinia amylovora</style><style face="normal" font="default" size="100%"> isolated from pear orchards in Washington State</style></title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>287–290</pages><volume>75</volume><dates><year>1991</year></dates><urls></urls></record></Cite></EndNote>] Similar to observations of streptomycin use, the quantity of oxytetracycline used for plant protection in the U.S. is much lower than the amounts permitted on the product labels.

Along with streptomycin, the U.S. EPA granted emergency use registrations for oxytetracycline on citrus in Florida and California to manage citrus greening. Usage data on oxytetracycline on citrus under these restricted emergency uses are not publically available at this time.

In addition to the U.S., Latin America permits use of oxytetracycline for crop protection (Table 6). Oxytetracycline is packaged either as a single antibiotic product or as antimicrobial combinations of oxytetracycline plus streptomycin or oxytetracycline plus streptomycin and copper. These formulations are used to manage fire blight on pome fruit in Mexico (Table 6). Oxytetracycline is also packaged and applied in combination with gentamicin and/or copper to manage diseases in flowers and vegetable crops in Latin America. The amount of oxytetracycline applied to crops in Latin American countries in not known.

Kasugamycin. Kasugamycin is a novel, structurally-unique aminoglycoside originally isolated from *Streptomyces kasugaensis* in Japan. Kasugamycin, also called kasumin, inhibits protein synthesis by a different mechanism than other aminoglycosides.[ADDIN EN.CITE <EndNote><Cite><Author>Yoshii</Author><Year>2012</Year><RecNum>12</RecNum><DisplayText><style face="superscript">[230]</style></DisplayText><record><rec-number>12</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971095">12</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Yoshii, Atsushi</author><author>Moriyama, Hiromitsu</author><author>Fukuhara, Toshiyuki</author></authors></contributors><titles><title>The Novel Kasugamycin 2’-N-Acetyltransferase Gene aac(2’)-IIa, Carried by the IncP Island, Confers

Kasugamycin Resistance to Rice-Pathogenic Bacteria</title><secondary-title>Applied and Environmental Microbiology</secondary-title></titles><periodical><full-title>Applied and Environmental Microbiology</full-title></periodical><pages>5555-5564</pages><volume>78</volume><number>16</number><dates><year>2012</year><pub-dates><date>August 15, 2012</date></pub-dates></dates><urls><related-urls><url>http://aem.asm.org/content/78/16/5555.abstract</url></related-urls></urls><electronic-resource-num>10.1128/aem.01155-12</electronic-resource-num></record></Cite></EndNote>]
Kasugamycin is used for control of bacterial diseases of rice, kiwifruit, walnuts, and fruit trees (Table 8).[22] Resistance to kasugamycin in plant pathogens occurs via spontaneous mutation in the *ksg* operon (dimethyltransferase) or 16S ribosomal RNA (16SrRNA), or through the modification by an acetyltransferase enzyme. Kasugamycin has no clinical or veterinary applications. There is no known cross-resistance between kasugamycin and aminoglycosides used in human medicine. In addition, kasugamycin resistance is not known to be linked to resistance to antibiotics used in human medicine. For these reasons, kasugamycin use as a pesticide is not currently considered a risk for the selection of resistance that affects human health. It is important to periodically monitor kasugamycin for cross-resistance and co-selection potential.

Gentamicin. Gentamicin is an aminoglycoside used to control several bacterial diseases of agave, vegetables, peppers, pear, rice, tomatoes, and tobacco in countries in Latin America. It is also an antibiotic commonly used in human medicine, including treatment of serious bacterial infections. According to product labels, gentamicin is not sold as a single antimicrobial product, but rather in combination with oxytetracycline or copper compounds (Table 6). The labels for products containing gentamicin were accessed on the website [HYPERLINK "http://www.terralia.com/agroquimicos_de_mexico/composition_index"]. To protect crops, products containing gentamicin are applied to fields between two to four times at 7-day intervals. The re-entry time into the treated areas often is listed as 12 hours after application. The labels did not specify pre-harvest interval consistently, except for pear, which is between 21 to 30 days depending on the product. Usage data on gentamicin in Latin American countries was not found.

Oxolinic acid. Oxolinic acid is a synthetic quinolone that inhibits the enzyme DNA gyrase. Oxolinic acid is related to fluoroquinolone antibiotics, which are commonly used in human medicine. Oxolinic acid has been used in Israel to control fire blight on pear since 1998, after streptomycin-resistant populations of *E. amylovora* emerged. The efficacy of oxolinic acid for fire blight control on pear has decreased over time, in part due to resistance to the antibiotic.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Oxolinic acid has been used in Japan and other countries to manage bacterial diseases of rice.[ADDIN EN.CITE <EndNote><Cite><Author>Hikichi</Author><Year>1989</Year><RecNum>429</RecNum><DisplayText><style face="superscript">[233, 234]</style></DisplayText><record><rec-number>429</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524144141">429</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hikichi, Y.,Noda, C., and Shimizu,K. </author></authors></contributors><titles><title>Oxolinic acid</title><secondary-title>Jpn. Pestic. Inf. </secondary-title></titles><periodical><full-title>Jpn. Pestic. Inf.</full-title></periodical><pages>21-23</pages><volume>55</volume><dates><year>1989</year></dates><urls></urls></record></Cite><Cite><Author>Maeda</Author><Year>2004</Year><RecNum>6</RecNum><record><rec-number>6</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971095">6</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Maeda, Yukiko</author><author>Kiba, Akinori</author><author>Ohnishi, Kouhei</author><author>Hikichi, Yasufumi</author></authors></contributors><titles><title>New method to detect oxolinic acid-resistant Burkholderia glumae infesting rice seeds using a mismatch amplification mutation assay polymerase chain reaction</title><secondary-title>Journal of General Plant Pathology</secondary-title></titles><periodical><full-title>Journal of General Plant Pathology</full-title></periodical><pages>215-217</pages><volume>70</volume><number>4</number><dates><year>2004</year><pub-dates><date>August 01</date></pub-dates></dates><isbn>1610-739X</isbn><label>Maeda2004</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1007/s10327-003-0114-3</url></related-urls></urls><electronic-resource-num>10.1007/s10327-003-0114-3</electronic-resource-num></record></Cite></EndNote>] It is not clear how many countries permit the use of oxolinic acid for disease management and which crops are treated.

Copper. Copper is the most widely used compound to manage bacterial and fungal plant diseases. Copper-containing crop pesticides are used on nearly every food crop, crops grown for animal feed, and ornamentals. As a crop pesticide, copper can be phytotoxic (harmful to plants) and cause damage, especially on newly growing shoots, leaves, and fruit surfaces. As a pesticide, there are concerns about accumulation of copper in soils resulting in phytotoxicity. Copper also has been shown to co-select for antimicrobial resistance. This subject has been widely reviewed.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Copper underwent a re-registration review by the U.S. EPA in 2017. EPA amended the product labels to include methods that reduce the potential for spray drift to non-target areas for ground and aerial applications. Additionally, a designated re-entry time for all copper-containing pesticides was set at 48 hours for field use and 24 hours for greenhouse use. Other statements related to potential environmental hazards, especially regarding toxicity to fish, aquatic invertebrates, and aquatic systems, were added to labels. Finally, maximum amounts of copper per application, reapplication intervals, and maximum annual rates of copper per acre were established for all crops. The summary of the decision is available at [HYPERLINK "https://www.regulations.gov/document?D=EPA-HQ-OPP-2010-0212-0061"]. See Appendix A for the actual use rates for copper on crops in the re-registration document cited above. The annual maximum rates of the copper ion permitted on food crops vary greatly from 1.2 kg/HA for cereal grains to 53 kg/HA for mango.

Estimates on copper use in the U.S. were obtained from the USGS database. The copper-component of crop pesticides varies among products. For example, copper may be included as metallic copper, copper hydroxide, copper octanoate, copper oxychoride, copper sulfate, or other forms. The usage data for copper-based pesticides are normalized to the amount of the copper (active ingredient) present in the product, and the total amount of copper used as crop pesticides was aggregated across formulations. Approximately 4,216,580 to 4,588,046 kg of copper was applied to plants in the U.S. in 2015.

The data from the USGS represents commercial farm use of copper. Copper containing products are also sold for residential use for disease control on garden, landscape plants, and moss control in lawns. Estimates of copper use by homeowners are not available.

Antifungals

At least 36 triazole agricultural fungicides exist, although only a subset are currently used in any given country. Most triazole fungicides end with the suffix "-azole;" however, several triazoles do not (e.g., myclobutanil, triadimefon, and flutriafol) and a few fungicides with that suffix belong to other fungicide classes (e.g., imidazoles, benzimidazoles). Certain agricultural triazoles (i.e., bromuconazole, difenoconazole, epoxiconazole, propiconazole, and tebuconazole) interact with *A. fumigatus* proteins in a way that is similar to medical triazoles, suggesting potential for cross-resistance, compared to other triazole fungicides tested (e.g., triadimefon).[ADDIN EN.CITE

<EndNote><Cite><Author>Snelders</Author><Year>2012</Year><RecNum>469</RecNum><DisplayTex
t><style face="superscript">[210]</style></DisplayText><record><rec-number>469</rec-
number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"
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type><contributors><authors><author>Snelders, Eveline</author><author>Camps, Simone M.
T.</author><author>Karawajczyk, Anna</author><author>Schaftenaar, Gijs</author><author>Kema,
Gert H. J.</author><author>van der Lee, Henrich A.</author><author>Klaassen, Corné
H.</author><author>Melchers, Willem J. G.</author><author>Verweij, Paul
E.</author></authors></contributors><titles><title>Triazole Fungicides Can Induce Cross-Resistance to
Medical Triazoles in Aspergillus fumigatus</title><secondary-title>PLOS ONE</secondary-
title></titles><periodical><full-title>PLoS One</full-
title></periodical><pages>e31801</pages><volume>7</volume><number>3</number><dates><year>2
012</year></dates><publisher>Public Library of Science</publisher><urls><related-
urls><url>https://doi.org/10.1371/journal.pone.0031801</url></related-urls></urls><electronic-
resource-num>10.1371/journal.pone.0031801</electronic-resource-
num></record></Cite></EndNote>]

Across countries, the U.S. has the most detailed publicly available data on triazole use in agriculture. According to the USGS Pesticide National Synthesis Project, which provides use estimates, total triazole use was over six times higher in 2015 than in 1992 (Figure 4). Estimates of triazole use were ~350-600 metric tons in 1992 and increased to ~2,600-3,750 metric tons in 2015 (preliminary estimates). Of the three triazoles used the most in 1992, two markedly declined in use: triadimefon (131 to 0.09 metric tons; high estimates) and myclobutanil (129 to 46 metric tons). The third most commonly used triazole in 1992, propiconazole, rose markedly (274 to 1,012 metric tons). It is estimated that several triazoles introduced since 1992 were the most heavily used in 2015: tebuconazole (1,256 metric tons), prothioconazole (412 metric tons), metconazole (217 metric tons), and difenoconazole (176 metric tons) (Figure 5).

In addition to triazoles applied in commercial agricultural settings by trained and certified applicators, there are available products to treat fungal diseases for home use (lawn and garden plants), including myclobutanil, propiconazole, tebuconazole and triticonazole. Information on use of triazoles by homeowners is not available.

Data from other countries are available through the FAOSTAT website of the Food and Agriculture Organization of the United Nations. This information is based on questionnaires submitted by member countries. In these data, triazoles are grouped with imidazoles (also known as diazoles) and cannot be identified separately. In the U.S., imidazole use was less than 1% that of triazole use in 2015. Of countries that reported data for 2014, the highest reported use of triazoles and imidazoles were in Ukraine (2,996 metric tons), Germany (2,705 metric tons), France (2,241 metric tons), the U.K. (1,430 metric tons), and Poland (1,230 metric tons). Triazole and imidazole use more than tripled between 2005 and 2014 in Poland and increased by 180% in Ukraine, 125% in the UK, and 70% in Germany (France did not report data in 2005). Further exploration of these data is needed, including adjustment for arable land area, particularly since Ukraine, Germany, and France reported triazole and imidazole use nearly as high as that of the U.S., which has a far larger land area.

Other Antimicrobial Compounds

In countries in Asia, other natural or synthetic antimicrobial products are used for crop protection. One example is Jingangmycin, which is validamycin A and synthesized by *Streptomyces* spp. Jingangmycin inhibits an enzyme called trehalase[ADDIN EN.CITE <EndNote><Cite><Author>Shigemoto</Author><Year>1992</Year><RecNum>413</RecNum><DisplayText><style face="superscript">[238]</style></DisplayText><record><rec-number>413</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524075800">413</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Shigemoto, Reiko</author><author>Okuno, Tetsuro</author><author>Matsuura, Kazuho</author></authors></contributors><titles><title>Effects of Validamycin A on the Growth of and Trehalose Content in Mycelia of <i>Rhizoctonia solani</i> Incubated in a Medium Containing Several Sugars as the Sole Carbon Source</title><secondary-title>Japanese Journal of Phytopathology</secondary-title></titles><periodical><full-title>Japanese Journal of Phytopathology</full-title></periodical><pages>685-690</pages><volume>58</volume><number>5</number><dates><year>1992</year></dates><urls></urls><electronic-resource-num>10.3186/jjphytopath.58.685</electronic-resource-num></record></Cite></EndNote>] and is used in Asia to control sheath blight in rice, which is caused by the fungal pathogen *Rhizoctonia solani*.[ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2015</Year><RecNum>4</RecNum><DisplayText><style face="superscript">[239]</style></DisplayText><record><rec-number>4</rec-number><foreign-

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08/04/received
08/10/revised
08/12/accepted</date></pub-dates></dates><publisher>The Korean Society of Mycology</publisher><isbn>1229-8093
2092-9323</isbn><accession-num>PMC4630442</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4630442/</url></related-urls></urls><electronic-resource-num>10.5941/MYCO.2015.43.3.339</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>]

Ningnanmycin is a synthetic pyrimidine nucleoside antimicrobial, and is used against viral plant diseases and fungal diseases like powdery mildew (label information at [HYPERLINK "http://www.cdxzy.com/en/proC/201209/156.html"]).

There are other antimicrobial materials that might be applied in different countries to protect crop plants, but little is known about the use of these materials. These compounds that do not have a recognized link for resistance to clinical antimicrobials, and are beyond the scope of this report.

B. When antimicrobials are used as pesticides, what is the exposure of people who consume the produce or people who work in or nearby the crop field? What is the risk from this exposure?

Many countries regulate pesticide use, including antimicrobials that are used as pesticides. These regulations vary by country. In some countries, there is little to no regulation. This section describes a brief summary of regulation strategies in the U.S. and Europe to assess the risk of exposure and to reduce the exposure of people to antimicrobials.

In the U.S., the EPA is the federal regulatory agency for materials applied to plants. Many countries have similar agencies to regulate which materials can be used for plant production. In the U.S., each active ingredient is registered for use on a specific crop or crop group. For example, the crop group 'Pome fruit' includes apple, crabapple, mayhaw, Asian pear, quince, Chinese quince, Japanese quince, and

European pear. A material registered for the Pome fruit group can be used on any of these plants. Other materials are registered for a single member of the Pome fruit group, like European pear, and would be restricted for use only on pear trees. Individual states may introduce additional restrictions on pesticide use that would only apply to their state.

Prior to granting a registration for a material for crop health, EPA evaluates the environmental impact and possible detrimental effects of the active ingredient and formulation materials at a proposed dose on humans, animals, insects, other non-target organisms, and aquatic systems. Additionally, EPA establishes the Maximum Residue Level (MRL), defined as the amount of a pesticide allowed to remain in or on the harvested crop. Product labels on the EPA-approved materials include instructions for use and limitations. For example, streptomycin and tetracycline, EPA requires use of protective clothing and equipment for workers applying streptomyicin, a re-entry restriction of 12 hours after application, and a pre-harvest interval that specifies the number of days of the last application before the crop is harvested. The specific use directions, precautions, and restrictions listed on the product labels for materials used on crops are legally binding.

European Union legislation guides the use and marketing of plant protection products (Regulation (EC) No1107/2009). Prior authorization is needed before plant protection products can be placed on the market. A dual system is in place where EFSA evaluates active substances (the active component used in plant protection products against plant diseases) and member states evaluate and authorize the products at the national level. An active substance is approved if it is proven safe, meaning the substance and its residues do not have immediate or delayed harmful effects on human and animal health, and do not have unacceptable effects on the environment, particularly to non-target species and biodiversity. Active substances are approved for 15 years. The applicant can ask for a renewal before the expiration date. EFSA is responsible for proposing MRL.

The exposure limits for pesticides are based on estimates for toxicity to humans, which are developed by studying toxicity in experimental animals. However, this testing does not include measuring the effect of antimicrobial pesticides on the microbiome when exposed to the drug. Little is known about possible effects of antimicrobial pesticides on the human microbiome of those who might be exposed. Further studies are needed.

EFSA defines acceptable exposure as:

- Acceptable Daily Intakes (ADI): an estimate of the amount of a specific substance in food for drinking water that can be ingested on a daily basis over a lifetime without an appreciable health risk
- Acute Reference Doses (ARfD): an estimate of a daily oral exposure for an acute duration
- Acceptable Operator Exposure Level (AOEL): the maximum amount of active substance to which the operator may be exposed without any adverse health effects

Both ADIs and ARfD values are based on no observed adverse effect level (NoAEL), defined as the greatest concentration or amount of a substance at which no detectable adverse effects occur in animal toxicology studies, divided by a safety factor. The safety factor is set at 100 to account for the differences between test animals and humans (factor of 10), as well as the possible differences in sensitivity among humans (another factor of 10). Aminoglycosides, tetracyclines, and quinolones are not approved for use as pesticides in Europe, but triazoles are used. ADI, ARfD and AOEL values as set by EFSA for triazoles are given in Table 9.

In Europe, it is challenging to assess exposures of bystanders and residents because there is a lack of data for modelling. Biomonitoring of European operators handling antimicrobial pesticides could provide more realistic exposure information, especially if the compounds or metabolites are measured in blood or serum. Some antimicrobial pesticides are available for residential use in home gardens. Product labels provide safe handling instructions but regulators rely on the consumer to read these labels and follow instructions for appropriate and safe use of the product.

C. To what extent do antimicrobials used as pesticides contaminate the environment surrounding the crop field? What measures are effective in limiting spread?

Examples of Antimicrobial Pesticides Detected in the Environment Surrounding the Crop Field

Antimicrobials are more commonly monitored in many environmental areas, but there is not a lot of data linking antimicrobials specifically to pesticide use. For example, oxytetracycline is frequently detected in waterways like agricultural watersheds and this is considered to be the result of its widespread use in food-producing animals.[ADDIN EN.CITE <EndNote><Cite><Author>Dungan</Author><Year>2017</Year><RecNum>414</RecNum><DisplayText><style face="superscript">[240]</style></DisplayText><record><rec-number>414</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524075879">414</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Dungan, R. S.</author><author>Snow, D. D.</author><author>Bjorneberg, D. L.</author></authors></contributors><titles><title>Occurrence of

Antibiotics in an Agricultural Watershed in South-Central Idaho</title><secondary-title>J Environ Qual</secondary-title></titles><periodical><full-title>J Environ Qual</full-title></periodical><pages>1455-1461</pages><volume>46</volume><number>6</number><dates><year>2017</year><pub-dates><date>Nov</date></pub-dates></dates><isbn>0047-2425 (Print)
0047-2425 (Linking)</isbn><accession-num>29293847</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/29293847</url></related-urls></urls><electronic-resource-num>10.2134/jeq2017.06.0229</electronic-resource-num></record></Cite></EndNote>] To date, no experimental data links oxytetracycline occurrence in nature to its use as a crop pesticide. Similar conclusions may be drawn for oxolinic acid and aminoglycoside antibiotics. With regards to triazole fungicides, propiconazole and tebuconazole were detected in streams across the U.S.[ADDIN EN.CITE <EndNote><Cite><Author>Battaglin</Author><Year>2011</Year><RecNum>415</RecNum><DisplayText><style face="superscript">[241]</style></DisplayText><record><rec-number>415</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524075929">415</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Battaglin, William A.</author><author>Sandstrom, Mark W.</author><author>Kuivila, Kathryn M.</author><author>Kolpin, Dana W.</author><author>Meyer, Michael T.</author></authors></contributors><titles><title>Occurrence of Azoxystrobin, Propiconazole, and Selected Other Fungicides in US Streams, 2005–2006</title><secondary-title>Water, Air, & Soil Pollution</secondary-title></titles><periodical><full-title>Water, Air, & Soil Pollution</full-title></periodical><pages>307-322</pages><volume>218</volume><number>1</number><dates><year>2011</year><pub-dates><date>June 01</date></pub-dates></dates><isbn>1573-2932</isbn><label>Battaglin2011</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1007/s11270-010-0643-2</url></related-urls></urls><electronic-resource-num>10.1007/s11270-010-0643-2</electronic-resource-num></record></Cite></EndNote>] These antifungals are widely used in agriculture, and, in that study, the occurrence was likely related to their use in upstream areas because concentrations of propiconazole at sampling sites correlated with estimates of the antifungal use in upstream drainage basins. Propiconazole and tebuconazole also were detected in surface waters in Switzerland,[ADDIN EN.CITE <EndNote><Cite><Author>Kahle</Author><Year>2008</Year><RecNum>19</RecNum><DisplayText><s

tyle face="superscript">[242]</style></DisplayText><record><rec-number>19</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971195">19</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kahle, Maren</author><author>Buerge, Ignaz J.</author><author>Hauser, Andrea</author><author>Müller, Markus D.</author><author>Poiger, Thomas</author></authors></contributors><titles><title>Azole Fungicides: Occurrence and Fate in Wastewater and Surface Waters</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>7193-7200</pages><volume>42</volume><number>19</number><dates><year>2008</year><pub-dates><date>2008/10/01</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/es8009309</url></related-urls></urls><electronic-resource-num>10.1021/es8009309</electronic-resource-num></record></Cite></EndNote>] which was suspected, though not confirmed, to originate from agricultural use or urban runoff rainwater. In another study, tebuconazole was detected in sediment and amphibian tissue samples from Yosemite National Park and other sites in California's Sierra Nevada mountains.[ADDIN EN.CITE <EndNote><Cite><Author>Smalling</Author><Year>2013</Year><RecNum>22</RecNum><DisplayText><style face="superscript">[243]</style></DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971195">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kelly L. Smalling</author><author>Gary M. Fellers</author><author>Patrick M. Kleeman</author><author>Kathryn M. Kuivila</author></authors></contributors><titles><title>Accumulation of pesticides in pacific chorus frogs (Pseudacris regilla) from California's Sierra Nevada Mountains, USA</title><secondary-title>Environmental Toxicology and Chemistry</secondary-title></titles><periodical><full-title>Environmental Toxicology and Chemistry</full-title></periodical><pages>2026-2034</pages><volume>32</volume><number>9</number><dates><year>2013</year></dates><urls><related-urls><url>https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.2308</url></related-urls></urls><electronic-resource-num>doi:10.1002/etc.2308</electronic-resource-num></record></Cite></EndNote>] Because this fungicide was not known to be used at those sites, but was heavily used in the downwind agricultural Central Valley, the researchers suspected airborne deposition. Overall, few studies have examined occurrence of triazoles in the environment despite a substantial increase in use in the U.S. since 2005 ([HYPERLINK "https://water.usgs.gov/nawqa/pnsp/usage/maps/compound_listing.php"]).

Ecological and human health risk assessments have relied mostly on predicting environmental concentrations based on modeling. For example, the EPA calculated upper bound concentrations of streptomycin or oxytetracycline that might be found in surface and ground waters due to their use on apple (aerial spray application scenario) (U.S. EPA streptomycin, 2006) or peach/nectarine orchards, respectively (U.S. EPA oxytetracycline, 2006). Modeling was also applied to obtain the worst-case global maximum epoxiconazole concentration (1.215 mg/L) for stream runoff.[ADDIN EN.CITE <EndNote><Cite><Author>Chambers</Author><Year>2014</Year><RecNum>15</RecNum><DisplayText><style face="superscript">[49]</style></DisplayText><record><rec-number>15</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971195">15</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Chambers, Janice E.</author><author>Greim, Helmut</author><author>Kendall, Ronald J.</author><author>Segner, Helmut</author><author>Sharpe, Richard M.</author><author>Van Der Kraak, Glen</author></authors></contributors><titles><title>Human and ecological risk assessment of a crop protection chemical: a case study with the azole fungicide epoxiconazole</title><secondary-title>Critical Reviews in Toxicology</secondary-title></titles><periodical><full-title>Critical Reviews in Toxicology</full-title></periodical><pages>176-210</pages><volume>44</volume><number>2</number><dates><year>2014</year><pub-dates><date>2014/02/01</date></pub-dates></dates><publisher>Taylor & Francis</publisher><isbn>1040-8444</isbn><urls><related-urls><url>https://doi.org/10.3109/10408444.2013.855163</url></related-urls></urls><electronic-resource-num>10.3109/10408444.2013.855163</electronic-resource-num></record></Cite></EndNote>] A model of triazole use on soybeans estimated that these antifungals would be present in field runoff and shallow groundwater in concentrations that exceed chronic human health exposure thresholds.[ADDIN EN.CITE <EndNote><Cite><Author>Deb</Author><Year>2010</Year><RecNum>417</RecNum><DisplayText><style face="superscript">[244]</style></DisplayText><record><rec-number>417</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524076215">417</key></foreign-keys><ref-type name="Journal Article">17</ref-

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Parameters Influencing the Mobility of Antimicrobials in the Environment

Several factors influence the environmental fate of a pesticide, such as their physicochemical properties (e.g., sensitivity to ultraviolet light degradation), their mode of application, soil and hydrological conditions, or climatic conditions. Compounds such as oxytetracycline and aminoglycosides are quite water-soluble (Royal Society of Chemistry, 2017), whereas triazoles are relatively less water-soluble. The range of factors suggest that there are differences in mobility and fate in the environment. For example when the pesticide is applied as spray, simulated heavy rainfalls removed oxytetracycline from the leaf surface within minutes.[ADDIN EN.CITE <EndNote><Cite><Author>Christiano</Author><Year>2010</Year><RecNum>418</RecNum><DisplayText><style face="superscript">[245]</style></DisplayText><record><rec-number>418</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524076250">418</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Christiano, R. S. C.</author><author>Reilly, C. C.</author><author>Miller, W. P.</author><author>Scherm, H.</author></authors></contributors><titles><title>Oxytetracycline Dynamics on Peach Leaves in Relation to Temperature, Sunlight, and Simulated Rain</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>1213-1218</pages><volume>94</volume><number>10</number><dates><year>2010</year><pub-dates><date>2010/10/01</date></pub-dates></dates><publisher>Scientific

Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS-04-10-0282</url></related-urls></urls><electronic-resource-num>10.1094/PDIS-04-10-0282</electronic-resource-num><access-date>2018/04/18</access-date></record></Cite></EndNote>]
However, when injected into the trunk of citrus trees, oxytetracycline residues could persist in the leaves and roots for months.[ADDIN EN.CITE
<EndNote><Cite><Author>Hu</Author><Year>2016</Year><RecNum>18</RecNum><DisplayText><style face="superscript">[39]</style></DisplayText><record><rec-number>18</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971195">18</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hu, Jiahuai</author><author>Wang, Nian</author></authors></contributors><titles><title>Evaluation of the Spatiotemporal Dynamics of Oxytetracycline and Its Control Effect Against Citrus Huanglongbing via Trunk Injection</title><secondary-title>Phytopathology</secondary-title></titles><periodical><full-title>Phytopathology</full-title></periodical><pages>1495-1503</pages><volume>106</volume><number>12</number><dates><year>2016</year><pub-dates><date>2016/12/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0031-949X</isbn><urls><related-urls><url>https://doi.org/10.1094/PHYTO-02-16-0114-R</url></related-urls></urls><electronic-resource-num>10.1094/PHYTO-02-16-0114-R</electronic-resource-num><access-date>2018/03/22</access-date></record></Cite></EndNote>]

Soil characteristics like pH, ionic strength, metal ions, and organic matter content influence the adsorption processes of antimicrobials and their mobility.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Recent studies seem to indicate that even though soil might adsorb a compound, it may still exert selective pressure on exposed bacteria, increasing risk that resistance might be developed.[ADDIN EN.CITE
<EndNote><Cite><Author>Chen</Author><Year>2017</Year><RecNum>16</RecNum><DisplayText><style face="superscript">[248]</style></DisplayText><record><rec-number>16</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971195">16</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Chen, Zeyou</author><author>Zhang, Wei</author><author>Wang, Gang</author><author>Zhang, Yingjie</author><author>Gao, Yanzheng</author><author>Boyd, Stephen A.</author><author>Teppen, Brian J.</author><author>Tiedje, James M.</author><author>Zhu, Dongqiang</author><author>Li,

Hui</author></authors></contributors><titles><title>Bioavailability of Soil-Sorbed Tetracycline to Escherichia coli under Unsaturated Conditions</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>6165-6173</pages><volume>51</volume><number>11</number><dates><year>2017</year><pub-dates><date>2017/06/06</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/acs.est.7b00590</url></related-urls></urls><electronic-resource-num>10.1021/acs.est.7b00590</electronic-resource-num></record></Cite></EndNote>] A better understanding of selective pressure of antimicrobials in soil systems is needed. [ADDIN EN.CITE <EndNote><Cite><Author>Gonsalves</Author><Year>1977</Year><RecNum>17</RecNum><DisplayText><style face="superscript">[249]</style></DisplayText><record><rec-number>17</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971195">17</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gonsalves, Dennis</author><author>Tucker, D. P. H.</author></authors></contributors><titles><title>Behavior of oxytetracycline in Florida citrus and soils</title><secondary-title>Archives of Environmental Contamination and Toxicology</secondary-title></titles><periodical><full-title>Archives of Environmental Contamination and Toxicology</full-title></periodical><pages>515-523</pages><volume>6</volume><number>1</number><dates><year>1977</year><pub-dates><date>December 01</date></pub-dates></dates><isbn>1432-0703</isbn><label>Gonsalves1977</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1007/BF02097790</url></related-urls></urls><electronic-resource-num>10.1007/bf02097790</electronic-resource-num></record></Cite></EndNote>]

Antimicrobial Persistence in the Environment

Studies on abiotic degradation, biotic degradation, and field dissipation are needed to understand the persistence and fate of pesticides in the environment. Compounds such as validamycin A may dissipate relatively quickly in soil, as illustrated in a study with controlled conditions, where residues became undetectable after 7 days of spray application.[ADDIN EN.CITE <EndNote><Cite><Author>Xu</Author><Year>2009</Year><RecNum>421</RecNum><DisplayText><style face="superscript">[250]</style></DisplayText><record><rec-number>421</rec-number><foreign-

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timestamp="1524076338">421</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Xu, Pengjun</author><author>Jiang, Shuren</author><author>Tao, Bu</author><author>Zhang, Hongyan</author></authors></contributors><titles><title>Determination and study on degradation dynamics of fungicide validamycin a residue in soil using pre-column derivatization and capillary gas chromatography</title><secondary-title>Journal of Analytical Chemistry</secondary-title></titles><periodical><full-title>Journal of Analytical Chemistry</full-title></periodical><pages>818-822</pages><volume>64</volume><number>8</number><dates><year>2009</year><pub-dates><date>August 01</date></pub-dates></dates><isbn>1608-3199</isbn><label>Xu2009</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1134/S1061934809080085</url></related-urls></urls><electronic-resource-num>10.1134/s1061934809080085</electronic-resource-num></record></Cite></EndNote>]
Other compounds may be more persistent. For example, oxytetracycline residues could still be detected in low concentrations in soil after one and a half years after infection into young non-bearing trees.[ADDIN EN.CITE
<EndNote><Cite><Author>Gonsalves</Author><Year>1977</Year><RecNum>17</RecNum><DisplayText><style face="superscript">[249]</style></DisplayText><record><rec-number>17</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"
timestamp="1523971195">17</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gonsalves, Dennis</author><author>Tucker, D. P. H.</author></authors></contributors><titles><title>Behavior of oxytetracycline in Florida citrus and soils</title><secondary-title>Archives of Environmental Contamination and Toxicology</secondary-title></titles><periodical><full-title>Archives of Environmental Contamination and Toxicology</full-title></periodical><pages>515-523</pages><volume>6</volume><number>1</number><dates><year>1977</year><pub-dates><date>December 01</date></pub-dates></dates><isbn>1432-0703</isbn><label>Gonsalves1977</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1007/BF02097790</url></related-urls></urls><electronic-resource-num>10.1007/bf02097790</electronic-resource-num></record></Cite></EndNote>] Based on the monitoring data in lakes, Kahle et al.[ADDIN EN.CITE
<EndNote><Cite><Author>Kahle</Author><Year>2008</Year><RecNum>19</RecNum><DisplayText><s

tyle face="superscript">[242]</style></DisplayText><record><rec-number>19</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971195">19</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kahle, Maren</author><author>Buerge, Ignaz J.</author><author>Hauser, Andrea</author><author>Müller, Markus D.</author><author>Poiger, Thomas</author></authors></contributors><titles><title>Azole Fungicides: Occurrence and Fate in Wastewater and Surface Waters</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>7193-7200</pages><volume>42</volume><number>19</number><dates><year>2008</year><pub-dates><date>2008/10/01</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/es8009309</url></related-urls></urls><electronic-resource-num>10.1021/es8009309</electronic-resource-num></record></Cite></EndNote>] also suggested that triazole compounds (fluconazole, propiconazole, and tebuconazole) may be relatively persistent in the aquatic environment.

Hydrolysis and photolysis—the breakdown of a compound due to reaction with water or by light, respectfully—are major mechanisms of abiotic degradation, and environmental factors (e.g., light exposure, pH, and temperature) could also influence their degradation.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Natural organic matter may also play a role in the fate of these compounds. For example, sorption on natural organic matter was shown to enhance phototransformation of aminoglycosides.[ADDIN EN.CITE <EndNote><Cite><Author>Li</Author><Year>2016</Year><RecNum>21</RecNum><DisplayText><style face="superscript">[252]</style></DisplayText><record><rec-number>21</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971195">21</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Li, Rui</author><author>Zhao, Cen</author><author>Yao, Bo</author><author>Li, Dan</author><author>Yan, Shuwen</author><author>O'Shea, Kevin E.</author><author>Song, Weihua</author></authors></contributors><titles><title>Photochemical Transformation of Aminoglycoside Antibiotics in Simulated Natural Waters</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title></periodical><pages>2921-

2930</pages><volume>50</volume><number>6</number><dates><year>2016</year><pub-dates><date>2016/03/15</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>https://doi.org/10.1021/acs.est.5b05234</url></related-urls></urls><electronic-resource-num>10.1021/acs.est.5b05234</electronic-resource-num></record></Cite></EndNote>]

Note that the disappearance of the parent compound does not correspond to a loss of antimicrobial activity. For example, the degradation products of streptomycin have shown residual antimicrobial activity.[ADDIN EN.CITE <EndNote><Cite><Author>Shen</Author><Year>2017</Year><RecNum>422</RecNum><DisplayText><style face="superscript">[251]</style></DisplayText><record><rec-number>422</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524076367">422</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Shen, Yanru</author><author>Zhao, Wenyan</author><author>Zhang, Chunling</author><author>Shan, Yujie</author><author>Shi, Junxian</author></authors></contributors><titles><title>Degradation of streptomycin in aquatic environment: kinetics, pathway, and antibacterial activity analysis</title><secondary-title>Environmental Science and Pollution Research</secondary-title></titles><periodical><full-title>Environmental Science and Pollution Research</full-title></periodical><pages>14337-14345</pages><volume>24</volume><number>16</number><dates><year>2017</year><pub-dates><date>June 01</date></pub-dates></dates><isbn>1614-7499</isbn><label>Shen2017</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1007/s11356-017-8978-5</url></related-urls></urls><electronic-resource-num>10.1007/s11356-017-8978-5</electronic-resource-num></record></Cite></EndNote>] The metabolites and degradation products of most antimicrobials have not yet been completely identified, so their impact on antimicrobial resistance remains mostly unknown. Progress in the field of mass spectrometry only recently allowed for the identification of some metabolites in crops and the environment. [ADDIN EN.CITE <EndNote><Cite><Author>Bauer</Author><Year>2018</Year><RecNum>14</RecNum><DisplayText><style face="superscript">[253]</style></DisplayText><record><rec-number>14</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971195">14</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bauer, Anna</author><author>Luetjohann, Jens</author><author>Hanschen, Franziska S.</author><author>Schreiner, Monika</author><author>Kuballa, Jürgen</author><author>Jantzen, Eckard</author><author>Rohn, Sascha</author></authors></contributors><titles><title>Identification and characterization of pesticide metabolites in Brassica species by liquid chromatography travelling wave ion mobility quadrupole time-of-flight mass spectrometry (UPLC-TWIMS-QTOF-MS)</title><secondary-title>Food Chemistry</secondary-title></titles><periodical><full-title>Food Chemistry</full-title></periodical><pages>292-303</pages><volume>244</volume><keywords><keyword>Pesticide metabolites</keyword><keyword>Suspect screening</keyword><keyword>Degradation</keyword><keyword>crops</keyword><keyword>UPLC-TWIMS-QTOF-MS</keyword></keywords><dates><year>2018</year><pub-dates><date>2018/04/01/</date></pub-dates></dates><isbn>0308-8146</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0308814617316072</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.foodchem.2017.09.131</electronic-resource-num></record></Cite></EndNote>]

Limiting the Spread of Antimicrobials

Pesticide product labels contain general recommendations from the suppliers, such as not applying directly to water, to areas where surface water is present, or to intertidal areas below the mean high water mark. Labels warn that using some of these chemicals may result in groundwater contamination in areas where soils are permeable, particularly where the water table is shallow. Recommendations also include:

- Not discharging equipment wash water or rinsate (diluted mixture of pesticides)
- Not applying when environmental conditions (e.g., wind) favor drift beyond the target application area
- Not exceeding a maximum number of applications per season
- Preventing livestock from grazing within the treated area
- Not applying close to or beyond the restricted days of harvest

Application methods recommended to avoid drift are based upon data-driven models. Prospective monitoring can help to ensure these measures effectively limit the spread of the parent compounds and their metabolites or degradation products.

D. To what extent do antimicrobials select for resistance within the crop field or surrounding environments? Is this resistance a threat to human health?

General Principles for Evaluating Risk from Current Crop Uses of Antimicrobials

Selection for resistance is primarily based on the length of exposure time and the concentration of the antimicrobial chemical that the microbial populations experience. Other factors that contribute to the likelihood of resistance selection are:

- The microbial population size (since emergence or natural occurrence of resistance is not a common event)
- Resources to amplify the resistance trait
- The ease at which the resistance-enabling trait occurs

The length of exposure is determined by how often the antimicrobial chemical is used and the stability of the chemical in the microbe's habitat, often expressed as half-life. Dissipation of antimicrobials can result from biodegradation by (resistant) microbes; photochemical transformation or chemical hydrolysis; loss by volatilization or co-distillation to the atmosphere; leaching away; and dilution by water. Most antimicrobials have a very low vapor pressure, so the loss by volatilization could be negligible. The concentration of the antimicrobial chemical that the microbes experience is also determined by the chemicals' bioavailability to the microbe (the amount that enters the cell and affects its critical functions). Bioavailability of many antimicrobials is reduced in soil due to their sorption to soil particles or organic matter, which reduces the selection for resistance. However, subinhibitory concentrations—those that are below the level capable of inhibiting microbe growth and replication—can have other effects, including inducing horizontal gene transfer which can confer resistance.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

The site where the antimicrobial chemical is applied can also substantially influence resistance selection. If the application is to leaves and immature fruits, which is how most antibiotics are applied, then the microbe exposure is relatively low because of the lower microbial density in these habitats and the higher potential for photochemical dissipation. Some antibiotics are injected into tree trunks, where microbial exposure is very low. In a relative sense, those application methods on crops would be predicted to experience much less resistance selection when compared to applying antibiotic-containing manures or recycled animal or urban waters to soil. In contrast to the antibiotics, triazole fungicides are applied broadly, including by aerial and ground spray application.

The selection of resistance in the environment also depends on the types of microbes present and the density of these organisms. It is common for environmental microbes to contain naturally occurring resistance mechanisms. The presence of an antimicrobial in the environment could result in the amplification of these resistant environmental bacteria. It is also possible for resistant genes in these bacteria to be mobilized into transferable genetic elements like plasmids. These mobile elements allow for resistance to move from one bacteria to another, a process also known as horizontal gene transfer. The following are necessary for horizontal gene transfer to occur among bacteria:

- The antibiotic resistance trait is on a mobile genetic element
- A high density of genetically related organisms present (since cell-cell contact and genetic compatibility are necessary)
- There is an available carbon source for the cell to complete its growth functions

The highest risk scenario is horizontal gene transfer of antibiotic resistance traits to a pathogen or to a commensal organism in the same environments as a human pathogen. Another high-risk scenario is the presence of human pathogens with mobile genetic elements in the environment from contamination of human waste or animal waste. In this case, the presence of the antimicrobial could amplify the resistant human pathogen. The application method for crop use of antibiotic would seem to provide negligible risk for this horizontal gene transfer scenario, but monitoring is needed, especially when the environment is contaminated with human pathogens.

The Threat of Resistance to Human Health

When evaluating the risk of using antimicrobials as pesticides and the potential to select for resistant microbes, antibiotics and antifungals should be considered separately because they are chemically distinct and target different types of microbes. For antibiotics, the very limited and special uses, apple and pear application, application to low density microbial habitats, and the low bioavailability would argue against the likelihood for significant resistance selection. The risk to human health from antibiotics applied on plants should be very low, and certainly so compared to the many other (non-crop) environmental sources for antibiotic resistance selection. Triazoles have a much larger, longer, and more diverse use and their stability in the environment would argue for much greater chances for resistance selection, which evidence supports. At present, the concern for antifungal resistance from agricultural fungicide use is largely restricted to *A. fumigatus*, but much remains unknown about other fungal clinical pathogens. For example, an important fungal disease caused by the yeast *Candida auris* has rapidly emerged in several world regions in the last few years. Most isolates of *C. auris* from ill people are resistant to the triazole fluconazole. More research is needed to understand the contribution of use of agricultural triazole fungicides to resistance in medically important fungi and yeasts.

E. How should environmental contamination of antimicrobials and emerging resistant bacteria be monitored?

Ongoing monitoring data using standardized methods are needed to address possible links between use of antimicrobial agents (i.e., selected antibiotics and triazoles) in agriculture and emergence of antimicrobial-resistant human pathogens.

Pesticide Use Data Globally

Publicly available data on use of selected antibiotics and antifungals (i.e., triazoles) in crop agriculture would allow researchers to target studies of antimicrobial resistance and evaluate geographic and temporal relationships between pesticide use and resistance. For many countries, data on use of these chemicals are limited or not available. To be most useful, use data should be provided for small geographic areas (e.g., county) and grouped by year and crop. Because available use data are provided in a wide range of formats, creation of a centralized data aggregation system could aid researchers.

Environmental Monitoring: Antimicrobials

Studies examining the persistence of selected antimicrobials and their metabolites are limited. Increased monitoring for these antimicrobials and their metabolites and degradation products in water, sediments, and other locations (e.g., air for triazoles) is needed to understand their environmental distribution. Monitoring animal wildlife for tissue concentrations with these antimicrobials might also be useful.[ADDIN EN.CITE <EndNote><Cite><Author>Smalling</Author><Year>2013</Year><RecNum>22</RecNum><DisplayText><style face="superscript">[243]</style></DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971195">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kelly L. Smalling</author><author>Gary M. Fellers</author><author>Patrick M. Kleeman</author><author>Kathryn M. Kuivila</author></authors></contributors><titles><title>Accumulation of pesticides in pacific chorus frogs (Pseudacris regilla) from California's Sierra Nevada Mountains, USA</title><secondary-title>Environmental Toxicology and Chemistry</secondary-title></titles><periodical><full-

title>Environmental Toxicology and Chemistry</full-title></periodical><pages>2026-
2034</pages><volume>32</volume><number>9</number><dates><year>2013</year></dates><urls><
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urls></urls><electronic-resource-num>doi:10.1002/etc.2308</electronic-resource-
num></record></Cite></EndNote>] Findings from such monitoring can be used in models to estimate distribution more widely. Triazoles in particular warrant further study, particularly given large increases in use over the past twenty years. Persistence of triazoles in the environment is often reported as days to weeks. However, triazoles may persist for months or longer in the environment, and environmental conditions heavily impact breakdown.[ADDIN EN.CITE
<EndNote><Cite><Author>Mosquera</Author><Year>2010</Year><RecNum>436</RecNum><DisplayTe
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181</pages><volume>75</volume><number>2</number><dates><year>2010</year><pub-
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database-name>PubMed</remote-database-
name><language>eng</language></record></Cite></EndNote>]

Environmental Monitoring: Antimicrobial Resistance

Monitoring for antimicrobial resistance in environmental bacteria and fungi isolated in and around agricultural environments is also needed. These data would optimally be collected in the same settings as antimicrobial concentration data and priority should be given to the detection of resistance in microbes that can cause disease. Data on antimicrobial resistance in bacteria and fungi that are not human pathogens may also be useful. For example, several *Aspergillus* species (e.g., *Aspergillus flavus*) are plant pathogens. Triazole fungicides are applied to grain crops to control leaf and stem diseases; generally, they are not used for control of ear or grain rots caused by *Aspergillus* spp. Nonetheless, the widely available data about triazole fungicide use on crops may be useful to generate estimates of incidental exposure of plant pathogenic *Aspergillus* spp. and emergence of resistance to triazoles. Triazole exposure data are not available for the human pathogen *Aspergillus fumigatus*.

Biomonitoring

Little is known about the concentrations of the selected antimicrobials in human populations resulting from environmental exposures. Small studies have examined urinary concentrations of the fungicide tebuconazole and its metabolites in occupational settings.[ADDIN EN.CITE <EndNote><Cite><Author>Fustinoni</Author><Year>2014</Year><RecNum>33</RecNum><DisplayText><style face="superscript">[256]</style></DisplayText><record><rec-number>33</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971730">33</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fustinoni, Silvia</author><author>Mercadante, Rosa</author><author>Polledri, Elisa</author><author>Rubino, Federico Maria</author><author>Mandic-Rajcevic, Stefan</author><author>Vianello, Giorgio</author><author>Colosio, Claudio</author><author>Moretto, Angelo</author></authors></contributors><titles><title>Biological monitoring of exposure to tebuconazole in winegrowers</title><secondary-title>Journal Of Exposure Science And Environmental Epidemiology</secondary-title></titles><periodical><full-title>Journal Of Exposure Science And Environmental Epidemiology</full-title></periodical><pages>643</pages><volume>24</volume><dates><year>2014</year><pub-dates><date>03/12/online</date></pub-dates></dates><publisher>Nature America, Inc.</publisher><work-type>Original Article</work-type><urls><related-urls><url>http://dx.doi.org/10.1038/jes.2014.14</url></related-urls></urls><electronic-resource-num>10.1038/jes.2014.14</electronic-resource-num></record></Cite></EndNote>] Systematic analysis of human samples collected via existing biomonitoring systems could provide insight into the degree and possible sources of exposure. Such analysis would need to distinguish on a population level between medical antimicrobial use and other exposures. Experience with biomonitoring for tobacco use via nicotine levels suggests that distinguishing between direct use of a product and environmental exposure is feasible.[ADDIN EN.CITE <EndNote><Cite><Author>Sexton</Author><Year>2004</Year><RecNum>34</RecNum><DisplayText><style face="superscript">[257]</style></DisplayText><record><rec-number>34</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971730">34</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sexton, Ken</author><author>L.Needham, Larry</author><author>L.Pirkle, James</author></authors></contributors><titles><title>Human Biomonitoring of Environmental Chemicals: Measuring chemicals in human tissues is the "gold standard" for assessing people's exposure to pollution</title><secondary-title>American Scientist</secondary-title></titles><periodical><full-title>American Scientist</full-title></periodical><pages>38-45</pages><volume>92</volume><number>1</number><dates><year>2004</year></dates><publisher>Sigma Xi, The Scientific Research Society</publisher><isbn>00030996</isbn><urls><related-urls><url>http://www.jstor.org/stable/27858331</url></related-urls></urls><custom1>Full publication date: JANUARY-FEBRUARY 2004</custom1></record></Cite></EndNote>]

Public Health Surveillance for Antimicrobial-resistant Infections

Although many factors influence antimicrobial resistance in human infections, public health surveillance for bacterial and fungal infections is essential for understanding the burden of resistance and for guiding studies examining links between environmental use of antimicrobials and resistant infections. Many examples of national and sentinel laboratory-based infectious disease surveillance exist. In the U.S. and Canada, no such broad-scale surveillance exists for *A. fumigatus* infections.

F. What strategies can be used to reduce or eliminate the need to use antimicrobials on crops?

By far, the best approach to limit the use of antimicrobials in plant production is through the use of the well-established measures of "Integrated Pest Management" (IPM), an approach designed to minimize economic losses to crops, as well as risks to people and the environment. The main components of IPM for plant diseases are:

- Accurate diagnosis and monitoring, which can also include disease modeling and predictive systems to guide the timing of plant protection product applications

- Use of disease resistant crop varieties, including resistant rootstocks in both fruit and vegetable systems
- Exclusionary practices that prevent the introduction of pathogens into a crop, such as using pathogen-free true seed and vegetative planting material, clean irrigation water, and sanitation practices that prevent the movement of pathogens from plant-to-plant and field-to-field
- Site selection and soil improvement to maximize plant health and minimize environmental factors that favor pathogens
- Crop rotation and other cultural practices to prevent pathogen buildup
- When available, use of biological and biorational products that demonstrate efficacy in controlling disease
- Judicious use of antibiotics and fungicides

Consequently, growers use multiple methods, in addition to antibiotics, to control bacterial plant diseases. Genetic resistance of host plants is the best method to control disease. This method is used to manage some bacterial diseases in vegetable and row crops. Unfortunately, for the destructive disease fire blight in pear and apple, breeding efforts have not yielded resistant fruiting cultivars.[ADDIN EN.CITE <EndNote><Cite><Author>Norelli</Author><Year>2003</Year><RecNum>450</RecNum><DisplayText><style face="superscript">[221]</style></DisplayText><record><rec-number>450</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145756">450</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Norelli, John L.</author><author>Jones, Alan L.</author><author>Aldwinckle, Herb S.</author></authors></contributors><titles><title>Fire Blight Management in the Twenty-first Century: Using New Technologies that Enhance Host Resistance in Apple</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>756-765</pages><volume>87</volume><number>7</number><dates><year>2003</year><pub-dates><date>2003/07/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS.2003.87.7.756</url></related-urls></urls><electronic-resource-num>10.1094/PDIS.2003.87.7.756</electronic-resource-num><access-date>2018/04/19</access-date></record></Cite></EndNote>] All commercial pear cultivars are very susceptible to fire blight. The 'Red Delicious' apple is tolerant of fire blight. Floral infections kill fruiting spurs, but the progression of the disease into stems was limited and the trees were not killed. Due to consumer demand, the 'Red Delicious' apple has been largely replaced by newer cultivars (e.g., 'Gala', 'Fuji', 'Honeycrisp', and others) that are susceptible to fire blight. Modern technologies, such as genomic sequencing, marker-assisted breeding, and genome editing, could hasten the development of disease-resistant tree fruits and stone fruits.[ADDIN EN.CITE <EndNote><Cite><Author>Norelli</Author><Year>2003</Year><RecNum>450</RecNum><DisplayText><style face="superscript">[221, 258]</style></DisplayText><record><rec-number>450</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145756">450</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Norelli, John L.</author><author>Jones, Alan L.</author><author>Aldwinckle, Herb S.</author></authors></contributors><titles><title>Fire Blight Management in the Twenty-first Century: Using New Technologies that Enhance Host Resistance in Apple</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>756-765</pages><volume>87</volume><number>7</number><dates><year>2003</year><pub-dates><date>2003/07/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS.2003.87.7.756</url></related-urls></urls><electronic-resource-num>10.1094/PDIS.2003.87.7.756</electronic-resource-num><access-date>2018/04/19</access-date></record></Cite><Cite><Author>Yang</Author><Year>2012</Year><RecNum>483</RecNum><record><rec-number>483</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527176447">483</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Yang, Nannan</author><author>Reighard, Gregory</author><author>Ritchie, David</author><author>Okie, William</author><author>Gasic, Ksenija</author></authors></contributors><titles><title>Mapping quantitative trait loci associated with resistance to bacterial spot (Xanthomonas arboricola pv. pruni) in peach</title></titles><volume>9</volume><dates><year>2012</year></dates><urls></urls><electronic-resource-num>10.1007/s11295-012-0580-x</electronic-resource-num></record></Cite></EndNote>] While genetic modification of pear and apple for fire blight resistance may be possible, certified organic growers could not grow these trees in their orchards. Furthermore, conventional growers may not invest in planting new orchards with genetically modified fruit trees without assurance that the fruit will be marketable and acceptable to consumers for decades into the future.

Cultural control methods are used routinely to manage bacterial diseases. For annual vegetable and row crops, cultural practices include crop rotation with plants that are not hosts for the bacterial disease of concern, using disease-free seeds and tubers, and soil solarization. For perennial crops, like fruit trees, crop rotations are not possible. For fruit trees, the geographical location of the orchard can reduce the disease pressure of fire blight. For example, in the early 1900s, the pear industry moved from the east coast of the U.S. to the western states, like California, Washington, and Oregon. The warm, humid weather with frequent rain during the summer months in the eastern U.S. were favorable for infections of pear flowers and subsequent infections of branches (shoot blight), resulting in complete loss of orchards.[ADDIN EN.CITE <EndNote><Cite><Author>Thomson</Author><Year>2000</Year><RecNum>472</RecNum><DisplayText><style face="superscript">[219]</style></DisplayText><record><rec-number>472</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527127177">472</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>S. V. Thomson</author></authors><secondary-authors><author>J. L. Vanneste </author></secondary-authors></contributors><titles><title>Epidemiology of fire blight</title><secondary-title>Fire blight: the disease and its causative agent, Erwinia amylovora</secondary-title></titles><dates><year>2000</year></dates><pub-location>Wallingford, UK</pub-location><publisher>CAB International</publisher><urls></urls></record></Cite></EndNote>] In the western states, the dry conditions during the summer months reduces the incidence of damaging secondary stem infections caused by the fire blight pathogen.

Additional cultural control methods for bacterial diseases of fruit trees include:

- Sanitation (removing diseased tissues and planting disease-free plants)
- Adjusting fertilizer applications or using plant growth regulators to maintain plant health and to reduce vigor and production of succulent shoots
- Drip irrigation to reduce wetting of foliage and fruit
- Pruning to maintain good airflow through the canopy
- Managing harmful insects that may spread bacteria or cause wounds that would serve as infection sites

While pear and apple growers use IPM practices for fire blight management, the practices are insufficient. Additional tools are needed to protect tree fruits.

Non-antibiotic Chemical Control Methods for Fire Blight Management

A mixture of hydrogen dioxide and peroxyacetic acid is a general biocide that can be used to control fungal and bacterial diseases, including fire blight. The mixture of hydrogen dioxide and peroxyacetic acid kills bacteria on contact, but has little residual activity. Commercial sources are available.

Lime sulfur can be applied to apple trees during bloom to reduce the number of flowers and, consequently, the number of flowers that could be infected by the fire blight pathogen. This material is not used on pear during bloom.[ADDIN EN.CITE <EndNote><Cite><Author>Johnson</Author><Year>2013</Year><RecNum>442</RecNum><DisplayText><style face="superscript">[259]</style></DisplayText><record><rec-number>442</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145316">442</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Johnson, K. B., and T. N. Temple</author></authors></contributors><titles><title>Evaluation of strategies for fire blight control in organic pome fruit without antibiotics</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>402-409</pages><volume>97</volume><number>3</number><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>] Copper compounds can be applied to dormant pear and apple trees and repeated during early bloom.[ADDIN EN.CITE <EndNote><Cite><Author>Psallidas</Author><Year>2000</Year><RecNum>456</RecNum><DisplayText><style face="superscript">[260, 261]</style></DisplayText><record><rec-number>456</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524150401">456</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Psallidas, P. G., and J. Tsiantos</author></authors></contributors><titles><title>Chemical Control of Fire Blight</title><secondary-title>Fire Blight: The Disease and Its Causative Agent, Erwinia amylovora</secondary-title></titles><pages>199-234 </pages><dates><year>2000</year></dates><pub-location>Wallingford, UK</pub-location><publisher>CAB International</publisher><urls></urls></record></Cite><Cite><Author>Elkins</Author><Year>2015</Year><RecNum>438</RecNum><record><rec-number>438</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145020">438</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Elkins, Rachel B.</author><author>Temple, Todd N.</author><author>Shaffer, Carolyn A.</author><author>Ingels, Chuck A.</author><author>Lindow, Steven B.</author><author>Zoller, Broc G.</author><author>Johnson, Kenneth B.</author></authors></contributors><titles><title>Evaluation of Dormant-Stage Inoculum Sanitation as a Component of a Fire Blight Management Program for Fresh-

Market Bartlett Pear</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>1147-1152</pages><volume>99</volume><number>8</number><dates><year>2015</year><pub-dates><date>2015/08/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS-10-14-1082-RE</url></related-urls></urls><electronic-resource-num>10.1094/PDIS-10-14-1082-RE</electronic-resource-num><access-date>2018/04/19</access-date></record></Cite></EndNote>] If copper is applied on pear and apple trees with young developing fruit, the fruit surfaces may be damaged due to phytotoxicity, resulting in spotted or misshapen fruit that have a reduced market value. New formulations of copper bactericides are less phytotoxic and can be used during late bloom to control fire blight with less potential for damaging fruit finish.[ADDIN EN.CITE <EndNote><Cite><Author>Elkins</Author><Year>2015</Year><RecNum>438</RecNum><DisplayText><style face="superscript">[261]</style></DisplayText><record><rec-number>438</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145020">438</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Elkins, Rachel B.</author><author>Temple, Todd N.</author><author>Shaffer, Carolyn A.</author><author>Ingels, Chuck A.</author><author>Lindow, Steven B.</author><author>Zoller, Broc G.</author><author>Johnson, Kenneth B.</author></authors></contributors><titles><title>Evaluation of Dormant-Stage Inoculum Sanitation as a Component of a Fire Blight Management Program for Fresh-Market Bartlett Pear</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>1147-1152</pages><volume>99</volume><number>8</number><dates><year>2015</year><pub-dates><date>2015/08/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS-10-14-1082-RE</url></related-urls></urls><electronic-resource-num>10.1094/PDIS-10-14-1082-RE</electronic-resource-num><access-date>2018/04/19</access-date></record></Cite></EndNote>]

Two additional chemicals, which are not bactericidal, can be used for fire blight management. Prohexadione calcium is a plant growth regulator that is registered for apple. Prohexadione calcium reduces shoot growth, which can reduce damaging secondary infections in succulent shoots caused by the fire blight pathogen. This damage is common in orchards exposed to humid summers and frequent rain, as in the eastern U.S.[ADDIN EN.CITE

<EndNote><Cite><Author>Norelli</Author><Year>2003</Year><RecNum>450</RecNum><DisplayText><style face="superscript">[221]</style></DisplayText><record><rec-number>450</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145756">450</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Norelli, John L.</author><author>Jones, Alan L.</author><author>Aldwinckle, Herb S.</author></authors></contributors><titles><title>Fire Blight Management in the Twenty-first Century: Using New Technologies that Enhance Host Resistance in Apple</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>756-765</pages><volume>87</volume><number>7</number><dates><year>2003</year><pub-dates><date>2003/07/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS.2003.87.7.756</url></related-urls></urls><electronic-resource-num>10.1094/PDIS.2003.87.7.756</electronic-resource-num><access-date>2018/04/19</access-date></record></Cite></EndNote>] Acibenzolar-S-methyl can reduce disease severity by inducing a natural process called systemic activated plant resistance. This chemical may be used therapeutically on infected trees by drenching the soil or painting the material on infected branches or trunks to reduce canker expansion.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Biological Control Agents for Fire Blight

In the western U.S., the widespread emergence of streptomycin-resistant populations of *E. amylovora* in apple and pear orchards has increased grower's interest in biological control of fire blight. [ADDIN EN.CITE <EndNote><Cite><Author>Loper JE</Author><Year>1991</Year><RecNum>475</RecNum><DisplayText><style face="superscript">[211, 220]</style></DisplayText><record><rec-number>475</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527166542">475</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Loper JE, Henkels MD, Roberts RG, Grove GG, Willett MJ, Smith TJ</author></authors></contributors><titles><title><style face="normal" font="default" size="100%">Evaluation of streptomycin, oxytetracycline, and copper resistance in </style><style face="italic" font="default" size="100%">Erwinia amylovora</style><style face="normal" font="default" size="100%"> isolated from pear orchards in Washington State</style></title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-

title>Plant Disease</full-title></periodical><pages>287–290</pages><volume>75</volume><dates><year>1991</year></dates><urls></urls></record></Cite><Cite><Author>Jones</Author><Year>2000</Year><RecNum>443</RecNum><record><rec-number>443</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145418">443</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Jones, A. L., and E. L. Schnabel</author></authors><tertiary-authors><author>J. L. Vanneste </author></tertiary-authors></contributors><titles><title>The development of streptomycin-resistant strains of Erwinia amylovora</title><secondary-title>Fire Blight: The disease and its causative agent, Erwinia amylovora</secondary-title></titles><section>235-251</section><dates><year>2000</year></dates><publisher>CAB International</publisher><urls></urls></record></Cite></EndNote>] The emergence of streptomycin resistance destabilized antibiotic-based disease management programs, resulting in periodic epidemics in which entire orchards were lost. Thousands of microbes were isolated from orchards and screened for their ability to suppress the growth of *E. amylovora* on flowers, which would interrupt a key stage in the disease cycle.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Additional studies focused on disease control mechanisms of potential biological control agents, and possible adverse effects to fruit quality from the biological control agents. [ADDIN EN.CITE ADDIN EN.CITE.DATA]

Currently, several biological control agents are registered by the U.S. EPA to prevent fire blight. Two *Bacillus*-based products are sold to manage fire blight. *Bacillus amyloliquefaciens* strain D747 registered for the control of fungal and bacterial diseases on numerous crops, including pear and apple. *Bacillus subtilis* strain QST 713 is sold as a spray-dried fermentation product containing the live organism and a mixture of lipopeptides produced in culture. The lipopeptides are essential for efficacy, and growth of the bacterium on plant surfaces is not required for disease control. Similar to the timing of antibiotic applications, this agent is applied just prior to predicted infection periods, but numerous applications are recommended for disease control.

Several other biological agents manage fire blight by a mechanism called pre-emptive exclusion.[ADDIN EN.CITE <EndNote><Cite><Author>Wilson</Author><Year>1993</Year><RecNum>452</RecNum><DisplayText><style face="superscript">[268]</style></DisplayText><record><rec-number>452</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"

timestamp="1524146048">452</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wilson, M., and S. E. Lindow</author></authors></contributors><titles><title>Interactions between the biological control agent Pseudomonas fluorescens strain A506 and Erwinia amylovora in pear blossoms</title><secondary-title>Phytopathology</secondary-title></titles><periodical><full-title>Phytopathology</full-title></periodical><pages>117-123</pages><volume>83</volume><number>1</number><dates><year>1993</year></dates><urls></urls></record></Cite></EndNote>] In pre-emptive exclusion, nutrients for pathogen growth are depleted by the biocontrol agent and the pathogen is excluded from sites for colonization and infection. The biocontrol agents must be applied during early to mid-bloom to give it time to grow to large population sizes prior to floral colonization by the pathogen. Three biological control agents that operate in part by pre-emptive exclusion are *Pseudomonas fluorescens* strain A506, *Pantoea agglomerans* strain E325, and *Aureobasidium pullulans* strains DSM 14940 and DSM 14941. In addition to pre-emptive exclusion, the *Pantoea agglomerans* product produces an uncharacterized secondary metabolite on flowers that is toxic to *E. amylovora*.[ADDIN EN.CITE <EndNote><Cite><Author>Pusey</Author><Year>2011</Year><RecNum>484</RecNum><DisplayText><style face="superscript">[269]</style></DisplayText><record><rec-number>484</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1527176853">484</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pusey, P. L.</author><author>Stockwell, V. O.</author><author>Reardon, C. L.</author><author>Smits, T. H. M.</author><author>Duffy, B.</author></authors></contributors><titles><title>Antibiosis Activity of Pantoea agglomerans Biocontrol Strain E325 Against Erwinia amylovora on Apple Flower Stigmas</title><secondary-title>Phytopathology</secondary-title></titles><periodical><full-title>Phytopathology</full-title></periodical><pages>1234-1241</pages><volume>101</volume><number>10</number><dates><year>2011</year><pub-dates><date>2011/10/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0031-949X</isbn><urls><related-urls><url>https://doi.org/10.1094/PHYTO-09-10-0253</url></related-urls></urls><electronic-resource-num>10.1094/PHYTO-09-10-0253</electronic-resource-num><access-date>2018/05/24</access-date></record></Cite></EndNote>]

An advantage of biological control agents is that, unlike antibiotics, they grow and spread among flowers; that is, the biocontrol bacteria spread from colonized flowers to newly opened flowers that may not have been protected by earlier chemical sprays.[ADDIN EN.CITE ADDIN EN.CITE.DATA] Well-timed applications of the bacterial biological control agents during bloom can significantly reduce the incidence of fire blight under low to moderate disease pressure.[ADDIN EN.CITE ADDIN EN.CITE.DATA]

Challenges to Implementing Biological Control

Using biological control agents requires grower education and changes in how they approach fire blight management. Instead of using traditional decision aids to determine the need for disease control measures and the timing of intervention, growers need to commit during early bloom to a biologically based disease control program to allow for the establishment and growth of the biological control agents prior to the arrival of the pathogen to flowers. Furthermore, growers need to apply the biological control agents during conditions that support growth of the organism.[59] A decision-aid to use biological control agents was developed to guide the timing of applications to maximize the potential for successful establishment and growth prior to the pathogen migrating to flowers.[ADDIN EN.CITE <EndNote><Cite><Author>Johnson</Author><Year>2004</Year><RecNum>441</RecNum><DisplayText><style face="superscript">[274]</style></DisplayText><record><rec-number>441</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145228">441</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Johnson, K. B.</author><author>Stockwell, V. O.</author><author>Sawyer, T. L.</author></authors></contributors><titles><title>Adaptation of Fire Blight Forecasting to Optimize the Use of Biological Controls</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>41-48</pages><volume>88</volume><number>1</number><dates><year>2004</year><pub-dates><date>2004/01/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS.2004.88.1.41</url></related-urls></urls><electronic-resource-num>10.1094/PDIS.2004.88.1.41</electronic-resource-num><access-date>2018/04/19</access-date></record></Cite></EndNote>]

The biological control agents generally work best in the western U.S. states where bloom progresses over one to three weeks and conditions are moderately warm to support growth of the organism. In other regions of the U.S., bloom occurs rapidly and environmental conditions during early bloom are

often too cold to support rapid growth of the biological control agents, which may decrease control efficacy.[ADDIN EN.CITE
<EndNote><Cite><Author>Sundin</Author><Year>2009</Year><RecNum>446</RecNum><DisplayText><style face="superscript">[275]</style></DisplayText><record><rec-number>446</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145577">446</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sundin, George W.</author><author>Werner, Nicole A.</author><author>Yoder, Keith S.</author><author>Aldwinckle, Herb S.</author></authors></contributors><titles><title>Field Evaluation of Biological Control of Fire Blight in the Eastern United States</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>386-394</pages><volume>93</volume><number>4</number><dates><year>2009</year><pub-dates><date>2009/04/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS-93-4-0386</url></related-urls></urls><electronic-resource-num>10.1094/PDIS-93-4-0386</electronic-resource-num><access-date>2018/04/19</access-date></record></Cite></EndNote>]

Another barrier to widespread adoption of this technology is the lack of consistent performance by the biological control agents across environments.[ADDIN EN.CITE
<EndNote><Cite><Author>Johnson</Author><Year>2013</Year><RecNum>442</RecNum><DisplayText><style face="superscript">[259, 275]</style></DisplayText><record><rec-number>442</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145316">442</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Johnson, K. B., and T. N. Temple</author></authors></contributors><titles><title>Evaluation of strategies for fire blight control in organic pome fruit without antibiotics</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>402-409</pages><volume>97</volume><number>3</number><dates><year>2013</year></dates><urls></urls></record></Cite><Cite><Author>Sundin</Author><Year>2009</Year><RecNum>446</RecNum><record><rec-number>446</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145577">446</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sundin, George W.</author><author>Werner, Nicole A.</author><author>Yoder, Keith S.</author><author>Aldwinckle,

Herb S.</author></authors></contributors><titles><title>Field Evaluation of Biological Control of Fire Blight in the Eastern United States</title><secondary-title>Plant Disease</secondary-title></titles><periodical><full-title>Plant Disease</full-title></periodical><pages>386-394</pages><volume>93</volume><number>4</number><dates><year>2009</year><pub-dates><date>2009/04/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS-93-4-0386</url></related-urls></urls><electronic-resource-num>10.1094/PDIS-93-4-0386</electronic-resource-num><access-date>2018/04/19</access-date></record></Cite></EndNote>] In some years or locations, the biological control agents perform well, but in other years they might fail to control disease.[ADDIN EN.CITE <EndNote><Cite><Author>Stockwell</Author><Year>2010</Year><RecNum>55</RecNum><DisplayText><style face="superscript">[273]</style></DisplayText><record><rec-number>55</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971749">55</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Stockwell, V. O.</author><author>Johnson, K. B.</author><author>Sugar, D.</author><author>Loper, J. E.</author></authors></contributors><titles><title>Control of Fire Blight by Pseudomonas fluorescens A506 and Pantoea vagans C9-1 Applied as Single Strains and Mixed Inocula</title><secondary-title>Phytopathology</secondary-title></titles><periodical><full-title>Phytopathology</full-title></periodical><pages>1330-1339</pages><volume>100</volume><number>12</number><dates><year>2010</year><pub-dates><date>2010/12/01</date></pub-dates></dates><publisher>Scientific Societies</publisher><isbn>0031-949X</isbn><urls><related-urls><url>https://doi.org/10.1094/PHYTO-03-10-0097</url></related-urls></urls><electronic-resource-num>10.1094/PHYTO-03-10-0097</electronic-resource-num><access-date>2018/03/22</access-date></record></Cite></EndNote>] Additionally, while excellent disease control is reported with *Aureobasidium pullulans*, the yeasts may cause russet or mark fruit finish on certain cultivars of pear and apple during cool, wet environmental conditions.[ADDIN EN.CITE <EndNote><Cite><Author>Johnson</Author><Year>2013</Year><RecNum>442</RecNum><DisplayText><style face="superscript">[259]</style></DisplayText><record><rec-number>442</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145316">442</key></foreign-keys><ref-type name="Journal Article">17</ref-

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409</pages><volume>97</volume><number>3</number><dates><year>2013</year></dates><urls></
urls></record></Cite></EndNote>] Russet damages the fruit finish, decreasing the fresh market value of the fruit. Consequently, some growers hesitate to use *Aureobasidium pullulans*, especially in orchards in regions with cool, wet spring weather. Additionally, *Aureobasidium pullulans* are sensitive to copper and many of the fungicides used to control scab, powdery mildew, and other fungal diseases in orchards. The incompatibility of this biological product with many fungicides adds an extra level of complexity for managing fruit orchards during bloom to fruit development.[ADDIN EN.CITE
<EndNote><Cite><Author>Johnson</Author><Year>2013</Year><RecNum>442</RecNum><DisplayText
><style face="superscript">[259, 275]</style></DisplayText><record><rec-number>442</rec-
number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt"
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type><contributors><authors><author>Johnson, K. B., and T. N.
Temple</author></authors></contributors><titles><title>Evaluation of strategies for fire blight control
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409</pages><volume>97</volume><number>3</number><dates><year>2013</year></dates><urls></
urls></record></Cite><Cite><Author>Sundin</Author><Year>2009</Year><RecNum>446</RecNum><r
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id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524145577">446</key></foreign-keys><ref-
type name="Journal Article">17</ref-type><contributors><authors><author>Sundin, George
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394</pages><volume>93</volume><number>4</number><dates><year>2009</year><pub-
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Societies</publisher><isbn>0191-2917</isbn><urls><related-urls><url>https://doi.org/10.1094/PDIS-

93-4-0386</url></related-urls></urls><electronic-resource-num>10.1094/PDIS-93-4-0386</electronic-resource-num><access-date>2018/04/19</access-date></record></Cite></EndNote>]

In summary, antibiotics have been used for decades to control two serious plant diseases–fire blight in pear and apple, and bacterial spot in peach and nectarine–without documented deleterious effects to the environment or animal and human health.[ADDIN EN.CITE <EndNote><Cite><Author>McManus</Author><Year>2014</Year><RecNum>48</RecNum><DisplayText><style face="superscript">[276]</style></DisplayText><record><rec-number>48</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1523971749">48</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>McManus, Patricia S.</author></authors></contributors><titles><title>Does a drop in the bucket make a splash? Assessing the impact of antibiotic use on plants</title><secondary-title>Current Opinion in Microbiology</secondary-title></titles><periodical><full-title>Current Opinion in Microbiology</full-title></periodical><pages>76-82</pages><volume>19</volume><dates><year>2014</year><pub-dates><date>2014/06/01/</date></pub-dates></dates><isbn>1369-5274</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S1369527414000605</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.mib.2014.05.013</electronic-resource-num></record></Cite></EndNote>] IPM practices have reduced the number of antibiotics applications needed to manage fire blight and bacterial spot. Antibiotics are applied primarily when warm weather coincides with full bloom in orchards with a recent history of disease in the orchard or nearby. If these conditions are not met, antibiotics are not applied. In the U.S., organic-certified growers are at the forefront of testing if antibiotic-free commercial fruit production is feasible because antibiotics registrations for organic pear and apple production were withdrawn in October 2014. Given that fire blight epidemics generally occur every 5 to 10 years within a fruit-producing region, the capacity to control diseases like fire blight without antibiotics will likely be tested within the coming decade.

Tables and Figures

Table 1. Major methods for the detection of resistant pathogens and resistance genes

Method	Target	Benefits	Limitations	Cost / Technical Requirements
Laboratory culture	Pathogens	• Quantitative • Can have high sensitivity • Detects phenotypic resistance • Determines MIC	• Limited to culturable organisms	Low / Low
Whole genome sequencing	Pathogens	• Can detect all known resistance genes • Links resistance gene to host organism	• Must culture organism first • Cannot predict MIC	Medium / High
qPCR	Genes	• Quantitative • Culture not required	• Limit of detections vary • Limited number of targets • Does not link gene to host organism	Medium / Medium
Metagenomics	Genes	• Can detect all known resistance genes Culture not required	• Limit of detection unknown • Does not reliably link gene to host organism	High / High

Table 2. Main Methods for Production of Antimicrobials

Manufacturing Processes	
Fermentation	Antibiotic-producing microorganisms are grown in large vats, generally in quantities of 100,000–150,000 liters of liquid growth medium. The manufacturer can maintain ideal levels of microorganisms and produce maximum yields by controlling the oxygen concentration, temperature, pH, and nutrient levels. Once fermentation is complete, the antibiotic is extracted and purified to a crystalline product. This is easier to achieve if the antibiotic is soluble in organic solvent, or it first needs to be removed by ion exchange, adsorption, or chemical precipitation.
Synthetic	Antibiotics are made synthetically in the lab. These include the quinolone class of antibiotics, of which nalidixic acid is often credited as the first to be discovered.
Semi-synthetic	Antibiotics are produced through a combination of natural fermentation and laboratory work to maximize, or get the most out of, production. The production process can be controlled to influence the efficacy of the drug, amount of antibiotics produced, and potency (strength) of the antibiotic. The process depends on the type of drug and its intended use.

Table 3. Proposed Assays and Metrics for Safe Discharge Limits

Assay	Reported Metric	Reference
Estimation of a safety limit from minimal inhibitory concentrations (MIC) distribution data obtain from standard antimicrobial susceptibility testing results of bacterial isolates.	Predicted No Effect Concentration for selection	Bengtsson-Palme, 2016[ADDIN EN.CITE <EndNote><Cite><Author>Bengtsson-Palme</Author><Year>2016</Year><RecNum>249</RecNum><DisplayText><style face="superscript">[103]</style></DisplayText><record><rec-number>249</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524056782">249</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bengtsson-Palme, Johan</author><author>Larsson, D. G. Joakim</author></authors></contributors><titles><title>Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation</title><secondary-title>Environment International</secondary-title></titles><periodical><full-title>Environment International</full-title></periodical><pages>140-149</pages><volume>86</volume><keywords><keyword>Antibiotic resistance</keyword><keyword>Emission limits</keyword><keyword>Minimal selective concentrations</keyword><keyword>Predicted no effect concentrations</keyword><keyword>Good manufacturing practice</keyword><keyword>Environmental risk assessment</keyword></keywords><dates><year>2016</year><pub-dates><date>2016/01/01/</date></pub-dates></dates><isbn>0160-4120</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0160412015300817</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.envint.2015.10.015</electronic-resource-num></record></Cite></EndNote>]
Measuring the effect of an antimicrobial on pairwise pairwise competition of	Minimum Selectable Concentration	Gullberg, 2011[ADDIN EN.CITE <EndNote><Cite><Author>Gullberg</Author><Year>2011</Year><RecNum>246</RecNum><DisplayText><style face="superscript">[29]</style></DisplayText><record><rec-number>246</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524056703">246</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gullberg,

bacterial strains (resistant/wild-type) growing in liquid culture, extrapolating the antibiotic concentration where strains grow equally well.		Erik</author><author>Cao, Sha</author><author>Berg, Otto G.</author><author>Ilbäck, Carolina</author><author>Sandegren, Linus</author><author>Hughes, Diarmaid</author><author>Andersson, Dan I.</author></authors></contributors><titles><title>Selection of Resistant Bacteria at Very Low Antibiotic Concentrations</title><secondary-title>PLOS Pathogens</secondary-title></titles><periodical><full-title>PLOS Pathogens</full-title></periodical><pages>e1002158</pages><volume>7</volume><number>7</number><dates><year>2011</year></dates><publisher>Public Library of Science</publisher><urls><related-urls><url>https://doi.org/10.1371/journal.ppat.1002158</url></related-urls></urls><electronic-resource-num>10.1371/journal.ppat.1002158</electronic-resource-num></record></Cite></EndNote>]
Measuring the effect of antimicrobials on a complex microbial biofilm community derived from sewage effluent in either a test tube or a flow-through system. Multiple endpoints are used to determine	Minimum Selectable Concentration Lowest Observed Effect Concentration No Observed Effect Concentration	Lundstrom, 2016[ADDIN EN.CITE ADDIN EN.CITE.DATA] Kraupner, 2018[ADDIN EN.CITE <EndNote><Cite><Author>Kraupner N</Author><Year>2018</Year><RecNum>465</RecNum><DisplayText><style face="superscript">[201]</style></DisplayText><record><rec-number>465</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1524666973">465</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kraupner N, Ebmeyer S, Bengtsson-Palmer J, Fick J, Kristiansson E, Flack C-F, Larsson JDG</author></authors></contributors><titles><title>Selective concentrations for ciprofloxacin resistance in Escherichia coli grown in complex aquatic bacterial biofilms</title><secondary-title>Environ Intl</secondary-title></titles><periodical><full-title>Environ Intl</full-title></periodical><dates><year>2018</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/j.envint.2018.04.029</electronic-resource-num></record></Cite></EndNote>]

effect of the antimicrobial including phenotypic resistance, taxonomic changes and selection for chromosomal or transferrable resistance		

Table 4. Cross-selection and Co-selection Properties of Antimicrobials Used as Pesticides

Antimicrobial as a Pesticide	Relationship to Antimicrobials Used in Human Medicine	Cross-selection or Cross-resistance to Antimicrobials Used in Human Medicine
Streptomycin & Gentamicin	Streptomycin and gentamicin are used in human medicine and are related to several other aminoglycosides that are commonly used to treat serious infections caused by both Gram-negative and Gram-positive bacteria, like	Streptomycin & gentamicin can select for plasmid-mediated resistance mechanisms that confer resistance to these drugs and to all aminoglycosides.

	amikacin, gentamicin, tobramycin, plazomycin.	
Oxytetracycline	A member of the tetracycline class of antimicrobials, these drugs are used in human medicine to commonly used to treat infections caused by both Gram-negative and Gram-positive bacteria.	There are several resistance mechanisms that confer cross-resistance among the tetracycline antimicrobials.
Kasugamycin	Kasugamycin is not used in human medicine and is structurally dissimilar to related drugs that are used in human medicine, like aminoglycosides.	There is no evidence for cross-resistance. There is also no evidence for co-selection. Kasugamycin resistance mechanisms do not select for resistance to aminoglycosides used in human medicine and resistance to aminoglycosides used in human medicine do not confer resistance to kasugamycin.
Oxolinic Acid	Oxolinic acid is a quinolone and is related to fluoroquinolones commonly used in human medicine, like ciprofloxacin and levofloxacin.	Quinolone resistance confers cross-resistance to fluoroquinolones[ADDIN EN.CITE <EndNote><Cite><Author>Barry</Author><Year>1984</Year><RecNum>526</RecNum><DisplayText><style face="superscript">[277]</style></DisplayText><record><rec-number>526</rec-number><foreign-keys><key app="EN" db-id="axsavsds6zr9x1ee9eax05eszzt59fza55dt" timestamp="1528993393">526</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Barry, A L</author><author>Jones, R N</author></authors></contributors><titles><title>Cross-resistance among cinoxacin, ciprofloxacin, DJ-6783, enoxacin, nalidixic acid, norfloxacin, and oxolinic acid after in vitro selection of resistant populations</title><secondary-title>Antimicrobial Agents and Chemotherapy</secondary-title></titles><periodical><full-title>Antimicrobial Agents and Chemotherapy</full-title></periodical><pages>775-777</pages><volume>25</volume><number>6</number><dates><year>1984</year><pub-dates><date>June 1, 1984</date></pub-dates></dates><urls><related-urls><url>http://aac.asm.org/content/25/6/775.abstract</url></related-urls></urls><electronic-resource-num>10.1128/aac.25.6.775</electronic-resource-num></record></Cite></EndNote>]

Copper	Copper is a heavy metal and unrelated to antimicrobials used in human medicine.	Copper has co-selection potential. Disease-causing bacteria can carry heavy metal resistance in plasmids (mobile genetic elements) along with resistance to antibiotics used in human medicine.
Triazoles	Triazoles are a class of fungicide related to azole antifungals commonly used to treat human fungal infections, like fluconazole and intraconazole.	Cross-resistance occurs between triazoles and azoles used in human medicine.

Table 5. Registered Uses of Streptomycin for Crop Protection in the U.S.

Crop	Disease (causal agent)	Provisions
Tree fruit[a]		
Apple	Fire blight (*E. amylovora*)	Begin 100 ppm sprays at early to full bloom, then every 4 to 7 days during bloom. Continue sprays every 7 to 14 days until 50 days before harvest. May apply 6 to 8 times after bloom.
Pear	Fire blight (*E. amylovora*)	Begin 100 ppm sprays at early bloom, then every 3 to 5 days during bloom. Continue sprays every 5 to 14 days until 30 days before harvest. May apply up to 15 times during the season.
Seedlings grown in greenhouses until transplanted to field		
Celery (Florida only)	Bacterial blight (*Pseudomonas cichorii*)	Apply at 200 ppm. First application at two-leaf stage, then at 4 to 5 day intervals until celery is transplanted in field.
Peppers, tomato	Bacterial spot (*Xanthomonas euvesicatoria, Xanthomonas perforans*) Bacterial Speck (*Pseudomonas syringae* pv. tomato)	Apply at 200 ppm. First application at two-leaf stage, then at 4 to 5 day intervals until transplanted in field.
Row Crops		
Potato	Soft rot black leg (*Pectobacterium* spp.)	Soak cut seed pieces in 100 ppm streptomycin for several minutes, then plant in field.

Tobacco	Blue mold (*Peronospora tabacina*) Wildfire (*Pseudomonas syringae* pv. *tabaci*)	Apply at 100 or 200 ppm when plants are in two-leaf stage or when disease appears. Repeat at 5 to 7 day intervals until plants establish in field. Option to continue applications at weekly intervals.
Ornamentals		
Apple, Pear, Cotoneaster, Pyracantha	Fire blight (*E. amylovora*)	Apply at 100 ppm in early bloom, then every 3 to 4 days. After bloom spray every 5 to 7 days until fruit are visible.
Cuttings: Chrysanthemum, Dieffenbachia	Bacterial wilt (*Erwinia* spp.) Bacterial stem rot (*Pseudomonas* spp.)	Soak cuttings in 50 ppm or 200 ppm streptomycin for 4 hours or 20 minutes, respectively. Plant in sterile potting medium.
Numerous plants (e.g., Carnation, Forsythia, Lilac, Philodendron)	Bacterial leaf rot (*Xanthomonas campestris*)	Apply at 200 ppm every 4 to 5 days. If symptoms present, remove rotted leaves and spray every 4 days.
Roses (New Jersey only)	Crown gall (*Agrobacterium* spp.)	Remove galled tissue, soak root system and cut surfaces of plant in 200 ppm streptomycin for 15 minutes and replant in clean soil.

[a] Please note that emergency approval for use of oxytetracycline and streptomycin for citrus trees is not included in this table.

Table 6. Antibiotics Used as Crop Pesticides in Countries in Latin America

Crop	Disease	Materials[§]						
		Gm + oTc	Gm + oTc + Cu	oTc	oTc + Cu	oTc + Sm	oTc + Sm + Cu	Sm
Agave	Soft rot	X[¥]	X	–	–	–	–	–
Apple	Fire blight	–	–	–	–	–	–	X
Asparagus, garlic, onion, scallion	Bulb rot and bacterial blight	X	–	–	–	–	–	–
Carnation	Bacterial spot	X	–	–	X	–	–	–
Celery	Bacterial blight	–	–	–	–	–	–	X*
Chrysanthemum	Soft rot	X	–	–	X	–	–	–
Cucumber, melons, and squash	Angular leaf spot and rot	X	X	–	X	–	–	–

Eggplant, chili, peppers, potato, tomato, and tomatillo	Bacterial leaf spot	X	X	–	X	–	–	X*
Ornamentals	Crown gall and fire blight	–	–	–	–	–	–	X
Pear	Fire blight	X	–	X	–	X	X	–
Potato	Black leg and bacterial wilt	X	–	–	–	–	–	X*
Rice	Bacterial blight	X	–	–	–	–	–	–
Tobacco	Bacterial wilt and wildfire	X	-	-	X	-	-	-

§ Single antimicrobials and packaged mixtures. Cu= copper, Gm=gentamicin, oTc=oxytetracycline, and Sm=streptomycin.
¥ X indicates material used on crop
– denotes material not listed for crop.
* Indicates application only to seed or tubers.

Table 7. Use of Antibiotics for Crop Protection in the U.S. in 2015

				Antibiotic use on crops in 2015[a]		
Crop	Bearing fruit acreage (HA)[b]	Target pathogen	Antibiotic	Average number of applications	Acreage treated (%)	Total active ingredient (kg)
Apple	136,358	*Erwinia amylovora*	Kasugamycin	1.2	4	590
			Oxytetracycline	1.5	18	6,033
			Streptomycin	1.9	26	15,241
Peach	43,797	*Xanthomonas arboricola* pv. *pruni*	Oxytetracycline	2.2	6	771
Pear	20,823	*E. amylovora*	Kasugamycin	1.3	8	181
			Oxytetracycline	2.9	49	5,216
			Streptomycin	3.2	16	1,315
Total use[c]	200,978		Kasugamycin	1.3	4	771
			Oxytetracycline	2.2	18	12,020
			Streptomycin	2.5	25	16,556

[a] Chemical use data of antibiotics applied on crops from 2015 Survey on USDA, NASS website [HYPERLINK "https://quickstats.nass.usda.gov/"].
[b] Land area in hectares (HA) from 2012 Census of Agriculture, USDA, NASS website.
[c] Total use is presented as the 1) average of number of applications of an antibiotic across crops 2) acreage treated was calculated as the sum of HA of each crop treated with an antibiotic divided by the sum of the total HA of the crops and 3) sum of total active ingredient applied across crops.

Table 8. Current Registered Uses of Kasugamycin in Canada, New Zealand, and the United States

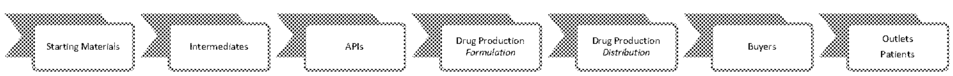
Crop, Country	Disease/causal agent
Cherry trees U.S.	Bacterial blast and bacterial canker (*Pseudomonas syringae* pv. *syringae*)
Fruiting vegetables (e.g., eggplant, peppers, tomatillo, tomato) Canada	Bacterial spot (*Xanthomonas campestris* pv *vesicatoria*) Bacterial stem canker (*Clavibacter michiganensis* spp *michiganensis*)
Kiwifruit vines New Zealand	Bacterial canker of kiwifruit (*Pseudomonas syringae* pv. *actinidiae*)
Pome fruit trees (e.g., apple and pear) Canada and U.S.	Fire blight (*Erwinia amylovora*)
Walnut trees U.S.	Walnut blight (*Xanthomonas campestris* pv. *juglandis*)

Table 9. ADI, ARfD, and AOEL Values for Triazoles as set by EFSA

Triazole	ADI, mg/kg body weight per day	ARfD, mg/kg body weight	AOEL, mg/kg body weight per day
Propiconazlole	0.04	0.3	0.1
Tebuconazole	0.03	0.03	0.03
Epoxiconazole	0.008	0.23	0.008
Difenoconazole	0.01	0.16	0.16
Bromuconazole	0.01	0.1	0.025

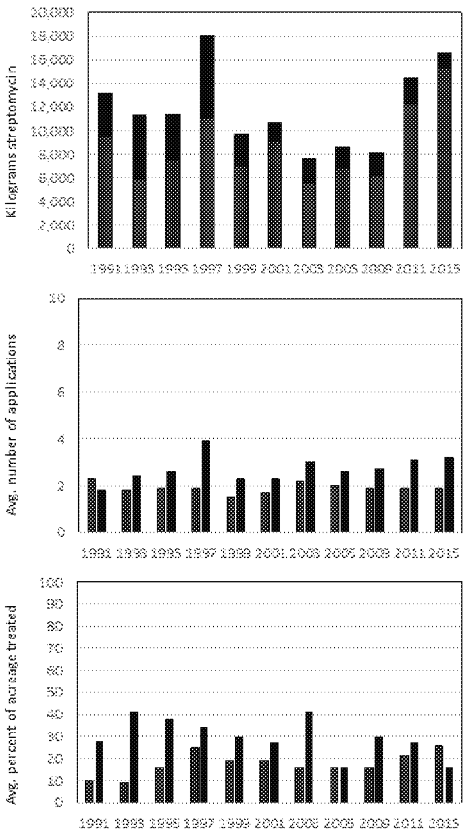
ADI, acceptable daily intake; ARfD, acute reference doses; AOEL, acceptable operator exposure level

Figure 1. Antimicrobials Supply Chain: A Complex Issue



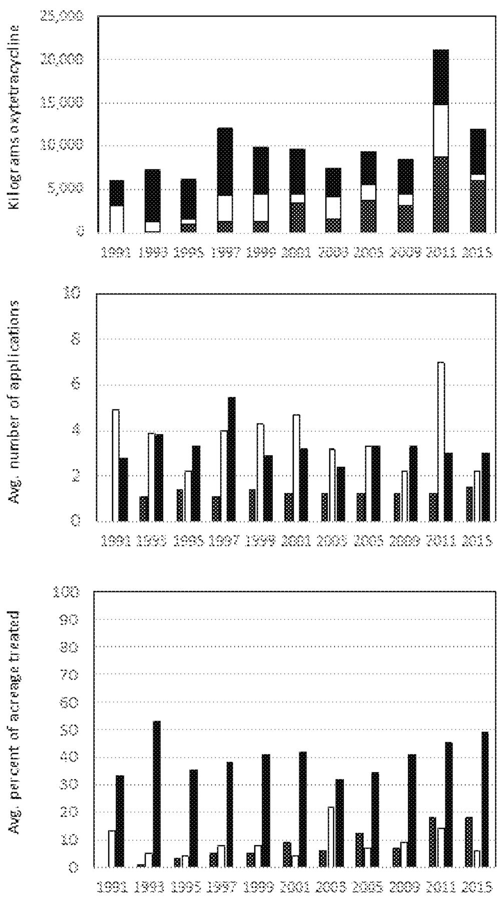
APIs: active pharmaceutical ingredients

Figure 2. Usage of Streptomycin on Apple (red bars) and Pear (black bars) in the U.S. (1991-2015)



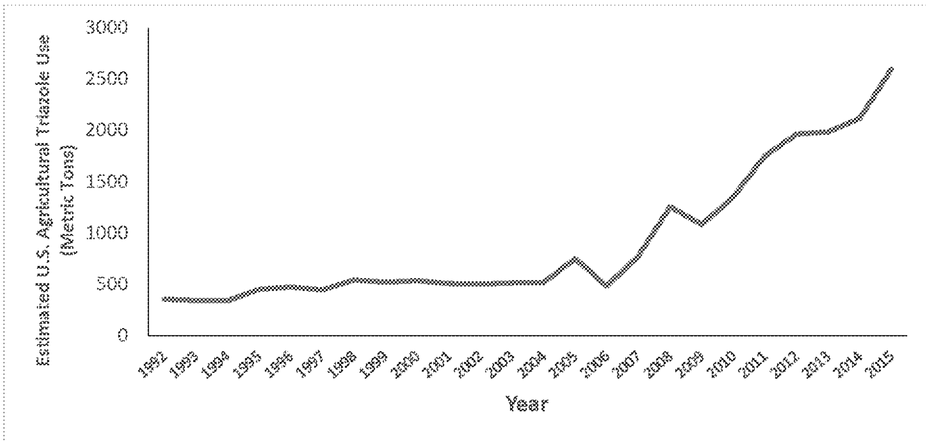
The upper graph is the total quantity of streptomycin in kilograms applied annually. The middle graph depicts the average number of applications of streptomycin on crops. The bottom graph shows the average percent of total acreage of a crop that was treated with streptomycin at least once.
Source: Usage data was obtained from USDA National Agricultural Statistics Service QuickStats database.

Figure 3. Usage of Oxytetracycline on Apple (red bars), Peach (white bars), and Pear (black bars) in the U.S. (1991-2015)



The upper graph is the total quantity of oxytetracycline in kilograms applied annually. The middle graph depicts the average number of applications of oxytetracycline on crops. The bottom graph shows the average percent of total acreage of a crop treated with oxytetracycline at least once.
Source: Usage data was obtained from the USDA National Agricultural Statistics Service QuickStats database.

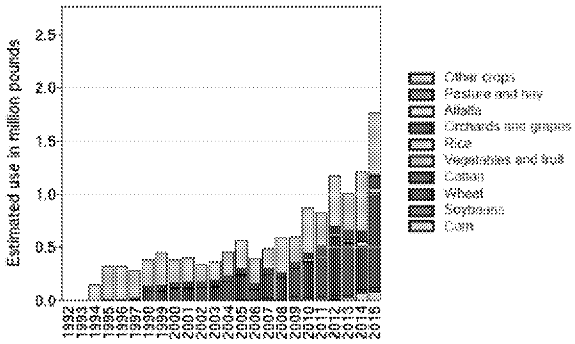
Figure 4. Estimates of Agricultural Triazole Fungicide Use by Year in the U.S.



Data for 2013–2015 are preliminary estimates that may be revised based on updated crop acreage data.
Data from 2015 do not include estimates for seed treatment applications.
Source: USGS Pesticide National Synthesis Project [HYPERLINK "https://water.usgs.gov/nawqa/pnsp/usage/maps/county-level/"]

Figure 5. Agricultural Tebuconazole Use by Year and Crop in the U.S.

Source: USGS Pesticide National Synthesis Project



[HYPERLINK "https://water.usgs.gov/nawqa/pnsp/usage/maps/county-level/"]

Literature Review

[ADDIN EN.REFLIST]

Glossary

Abiotic degradation: The breakdown of substances by chemical processes rather than by living organisms.

Active pharmaceutical ingredients (APIs): The biologically active substances within medicines (like antimicrobials) that have an effect on the patient (human or animal).

Adaptive immune system: The part of the immune system in humans and animals that eliminates pathogens or prevents their growth.

Adsorption: The binding of molecules from a gas or liquid to a solid surface.

Aerobic digestion: A bacterial sewage treatment process designed to reduce the volume of sewage sludge by adding oxygen, which allows microbes to grow and consume organic matter.

Amplification: An increase in the presence of antimicrobial-resistant bacteria or fungi in a reservoir or the environment.

Anaerobic digestion: The process of microbes breaking down materials without oxygen.

Antimicrobial resistance (AMR): When microbes develop the ability to reduce or eliminate the effectiveness of drugs, chemicals, or other agents used to cure or prevent infections. That means the microbes are not killed and continue to grow.

Aquaculture: The breeding, rearing, and harvesting of fish, shellfish, plants, and other organism in all types of water environments.

Biotic degradation (biodegradation): The process of breaking down organic substances by living microbes such as bacteria and fungi. This process can occur in surface water, sediment, and soil.

Biocide: A chemical or biological product that is intended to destroy, prevent the action of, or control a harmful microbe.

Bioconcentrate: The process of chemical accumulation in an organism.

Biosolids: Nutrient-rich organic materials produced from wastewater treatment facilities that can be applied to crops as fertilizer. Also called sewage sludge.

Cellular bioassay: A biochemical test that can be used to test the effect of antimicrobials on cells of a microorganism like bacteria for fungi.

Cephalosporinase: An enzyme produced by many species of bacteria that disrupt the beta-lactam ring of penicillin and cephalosporin classes of antibiotics and eliminates their effectiveness.

Antimicrobial stewardship: The use of antibiotics only when they are needed to treat disease, and to choose the right antibiotics and to administer them in the right way in every case.

Colonization: The presence of pathogens in the body without making a person sick.

Commensal: A relationship where one species benefits while the other is unaffected.

Contamination: The introduction of a harmful or foreign substance into an environment. This report uses contamination to describe antibiotics and AMR germs entering the environment when it would not naturally happen.

Driver: External factors that can lead to or amplify antimicrobial resistance, such as overuse of antimicrobials or transmission of resistant infections.

Ecotoxic: Chemical, physical, or biological stressors that are toxic to ecosystems or the environment.

Effluent: The liquid waste or sewage discharged into a waterway.

Electrochemical degradation: A wastewater treatment process that oxidizes organic compounds.

Enteric: The gut or small intestine.

Environment: The natural world or surroundings, including air, water, and soil. This report focuses its attention on water, sediment, and soil.

Epimerize: A chemistry term to describe a molecule changing forms.

Fallowing: An agricultural practice that calls for gaps in re-stocking fish pens to allow sediment to undergo natural recovery.

Fenton oxidation: The use of Fenton's reagent (a solution of hydrogen peroxide with ferrous iron) to create free radicals to oxidize a compound.

Functional genomics: The use of genomic data to study gene and protein expression and function on a genome-wide or system-wide level.

Gene amplification: An increase in the number of copies of a gene.

Grey water: The wastewater generated in households or buildings that has not come into contact with feces.

Horizontal gene transfer: The movement of genetic material directly from one organism to another, rather than between parent and offspring.

Human microbiome: The community of naturally-occurring microbes that live in or on the body (for example, stomach, intestines, skin). Antibiotics impact the microbiome by wiping out the natural composition of microbes. With a disrupted microbiome, resistant pathogens can take over and the body is less able to defend against infection, putting people at risk for potentially untreatable illnesses.

Hydrolyze: The use of electricity to separate water molecules into hydrogen and oxygen atoms.

Integrated Pest Management (IPM): An effective and environmentally sensitive management practice that uses information on pest life cycle in combination with available pest control methods to minimize possible risks to people and the environment.

Manure amendments: The addition of manure to soil to improve its physical or chemical properties. These additives can harbor pathogens.

Matrix: The components of a sample other than the compounds that are being targeted for analysis.

Metagenomics: The study of genetic material recovered from microbial communities in environmental samples.

MIC creep: The gradual increases in the lowest concentration of an antibiotic that prevents growth of a bacteria or fungi (minimum inhibitory concentration). MIC increase indicates that a microbe is developing reduced susceptibility or resistance to an antimicrobial.

Mobile genetic elements: The segments of DNA that can facilitate the movement of genetic material between bacterial chromosomes and can help spread resistance genes from one bacteria to another.

Mobilized resistance determinants: Resistance genes that are found on plasmids.

Mycelial mats: The vegetative part of a fungus that absorbs nutrients from the environment.

Neutralization: The process of adjusting the pH of a waste stream so that it is not too acidic or too basic before being discharged.

Non-pathogenic bacteria: Bacteria that do not cause disease, harm, or death to a host.

Ozonation: A water treatment process that introduces ozone into wastewater to destroy microorganisms and degrade pollutants.

Partitioning: A wastewater treatment process that separates components of a waste stream.

Pathogen: Organisms that cause disease in a host, like humans or animals.

Piscirickettsiosis: A disease affecting salmon, trout, and seabass that is caused by the bacteria *Piscirickettsia salmonis*.

Reservoir: A person, animal, insect, plant, or other host that is carrying a pathogen (for example, bacteria or fungi) that causes infectious diseases. Some pathogens have animal reservoirs (to survive, they need animal hosts). Others pathogens have human reservoirs (to survive, they need human hosts). This report discusses how water, sediment, and soil can act as a reservoir carrying antibiotic residue and resistant pathogens or genes.

Resistome: The collection of all the antimicrobial resistance genes in both pathogenic and non-pathogenic bacteria.

Reverse-osmosis: A water treatment technology that uses a filter or membrane to remove contaminants.

Sediment: Solid material (for example, rocks and minerals) that is broken down and moved by weathering and erosion, and are eventually deposited as a layer of solid particles on the bed or bottom of a body of water or other liquid.

Selective pressure: Any external factor that reduces reproductive success in a population.

Soakaways: A hole dug into the ground and filled with coarse stones that allows surface water to filter through the stones and into the ground.

Solid-phase extraction: A sample preparation process where compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties.

Sorption: A physical and chemical process where one substance attaches to another.

Volatilization: The process of evaporation and movement of pesticide vapors through the air.

Wastewater: Used water from fixtures like sinks and toilets that includes human waste and other substances like food scraps or soaps. In some cases, wastewater can also include storm water runoff. If wastewater is not properly treated, then the environment and human health can be negatively impacted.

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